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SOME OBSERVATIONS UPON THE AGGLUTINATION OF BACTERIA.*

WILLIAM HALLOCK PARK.

MY purpose in this address is to give briefly some observations upon the value of the agglutination test in establishing the identity or relationship of bacteria and in detecting the variety of bacteria exciting disease in cases of bacterial infection.

During the past three years, in connection with Dr. Katharine R. Collins, I have been more or less occupied in the study of these questions, and in this time I have learned much concerning the difficulties of properly interpreting the results, and of the limitations to the value of the agglutination reaction. It is my hope that a review of some of these experiences may be of interest. Before taking up the discussion of the two topics I wish to touch on some of the points to be thought of in the technique of carrying on the tests.

SOME IMPORTANT POINTS TO CONSIDER IN MAKING AN AGGLUTINATION TEST.

1. The quantitative nature of the union between bacteria and agglutinin. This necessitates that with increase in the number of bacteria in the serum dilution there is more material to combine with the agglutinin. A thick emulsion of bacteria is therefore not agglutinated in as high dilutions as a thin emulsion.

2. The varying sensitiveness of the same variety of bacteria from day to day, even when grown from the same stock culture. At

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*Address of Chairman of the Laboratory Section American Public Health Association.

times no explanation can be given for this variation, but of its occurrence there can be no doubt.

3. The increased rapidity of union of bacterial substance with agglutinin as the temperature rises from 0° to 37° C. This necessitates that not only the time at which readings are made should be stated, but also whether the reaction took place at the temperature of the ice box, the room, or the incubator.

4. The greater height of the reaction, when long rather than very short periods of time are allowed for its development, provided the test is so carried out that the bacteria do not multiply in the agglutinating fluid.

5. The absence of reaction at times in low dilutions, with presence of reaction in higher dilutions. This phenomenon appears to be due to substances in the serum other than agglutinins. It rarely occurs in dilutions of serum above 1:50.

6. The growth of some varieties of bacteria in the serum-dilutions when the temperature allows of it, thus altering the proportion between bacterial substance and agglutinin. A good reaction may thus disappear in the course of a few hours.

7. The individual judgment in the estimation of what constitutes a certain degree of reaction. No two observers read the completeness of a reaction exactly alike.

8. The alteration in the test serum when it is used over considerable periods of time. A gradual deterioration takes place in the agglutinin in the serum. This is more rapid in diluted serum and with increase of temperature.

9. The medium in which the bacterial suspension is made, whether broth or salt solution, whether it contains sugars or not, etc., is of importance. Growth in glucose media, for instance, makes bacteria more sensitive and tends to natural agglutination. Broth as a medium for diluting serum gives usually a quicker agglutination than does salt solution.

10. The effect of heat and of some preservatives, when they are used, in altering the serum both quantitatively and qualitatively.

11. The considerable difference in the readings made macroscopically and microscopically. A most striking example of the difference in reading is seen in the method used by Dunham[1] and

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[1] *Jour. Infect. Dis.*, 1906, Supplement No. 2. p. 10.

that by us. With meningococci examined microscopically after three hours in the incubator we obtained readings of a reaction of 1:200, while Dunham, using light suspensions in the ice chest for 36 hours, obtained a reading of 1:2,000. Both methods were probably equally correct, and the readings by one method could be compared with each other but not with those of the other method.

12. The observation, during the time of experiment, of the control specimen, and of any tendency to natural agglutination.

13. In absorption tests, when filtration methods are employed, the obstruction to the passage of agglutinins of the filter and of the bacteria coating the filter must be fully allowed for, as the coating formed by the various varieties of bacteria differs greatly in permeability.

AGGLUTINATION CHARACTERISTICS AS A GUIDE TO THE CLASSIFICATION AND IDENTIFICATION OF BACTERIA.

It has been unmistakably demonstrated that an agglutinating serum is composed of a number of agglutinins which owe their origin in the animal to the stimulus of the different proteid substances contained in a single cell or in several varieties of cells.

We have many facts which serve to point out the value of partially similar agglutination among bacteria in suggesting relationship such as between certain members of the typhoid-colon group of bacilli. It is true, however, that others which appear just as nearly related do not react to common agglutinins, and some that appear utterly unlike do react.

Thus Durham injected two animals with a different paratyphoid organism. These were obtained from two cases simulating typhoid fever and had the same biochemical activities. He found sera obtained from the two rabbits to have almost no similarity in agglutination. A serum clumping one 1:20,000 did not affect the other in dilutions of 1:100. The marked dissimilarity in the agglutinating characteristics of the bacilli contained in the colon group is another example. Among 14 strains of culturally characteristic colon bacilli isolated by us from 10 persons there were five distinct varieties, if classification were to be made by the agglutinating characteristics. In our recent investigations of pneumococci we have obtained a number of cultures from the exudate of characteristic cases of lobar pneumonia, which have been alike in morphology, in action on inulin, on sugars, and in cultural characteristics, and yet they have differed absolutely in their affinity for agglutinins. This difference remains unaltered in the cultures as they are continued on culture media, and undoubtedly indicates a different chemical composition; but this is too intangible to be a sufficient reason for separating bacteria which appear to be alike in more essential points. At times, however, it may be very instructive. Thus in Dr. Goodwin's paper on p. 21 it is noted that from the nasal secretion of two healthy students a diplococcus was isolated, which, except in its agglutination, appears to be identical with typical meningococci derived from spinal fluid. This difference in the diplococcus excludes it from the type obtained from the epidemic cases, and even places it under suspicion as to whether it is a meningococcus at all. The complete identity in agglutination characteristics between organisms obtained from the nasal cavity of the sick and from the spinal fluid is strong proof of the former being not only meningococci but the same identical variety as that in the cord.

A species of pathogenic bacteria which develops only in disease is apt to give rise to later generations, all of which will be alike in their agglutinating characteristics, while one which has for the most part a saprophytic life is apt to give rise to distinct varieties. Under semisaprophytic existence the new generations are subjected to variable conditions, and thus become modified, so that, as in the case of the pneumococci, the streptococci, and the colon group of bacilli, we may have a continuance of the more striking cultural characteristics with such variation in the agglutinating affinities as apparently to call for separation into numerous varieties. This separation is useless, so far as we can now see, for any practical purpose, and impossible to define, as there would be no way for future investigators to compare their results unless the original culture or the specific serum was at hand.

The stimulation in an animal of agglutinins for any microorganism is produced, according to our present views, by similar protoplasm in the infecting organisms. Some recent observations, if correct, indicate that cells apparently widely separated have more or less common substances. Ballner states that a rabbit immunized with a pink yeast developed agglutinins for both typhoid and dysentery bacilli, so that they were agglutinated in dilutions of 1:1,000. A rabbit, immunized by us with yeasts, developed a serum which agglutinated paradysentery bacilli but neither typhoid bacilli or true dysentery bacilli. A less striking experience of our own was that of the serum of a horse, which after immunization with a paradysentery bacillus agglutinated both that bacillus and a typical colon in dilutions of 1:10,000. During injections specific agglutinins are first chiefly developed, but later the total amount of group agglutinins increases so as, at times, to equal the specific ones. The proportional amounts of group agglutinins for allied bacteria differed greatly at different times during the immunization of an animal and at the same time in different animals. Many conflicting statements are due to the lack of appreciation of this variability.

As the quantitative agglutination test usually fails to distinguish whether the reaction is due to specific or group agglutinins, use has been made of an absorption method to determine the action of the specific agglutinin which is present among the multiple agglutinins in the serum of every immunized animal. It has been fairly established that any bacterial strain which can absorb from a serum all the agglutinins which acted upon a certain microorganism, and which were stimulated by that microorganism, must be identical with, or extremely closely allied to, it. The virulence of the two microorganisms may, however, vary widely.

The technique of making the absorption test is rather difficult. When the agglutinating strength of the serum is high, large amounts of bacteria must be added again and again, or the serum must be highly diluted. In the latter case it is impossible to demonstrate that the absorption is complete. Usually the serum is diluted with four times its quantity of salt solution, and then mixed with about its weight of culture. After standing a few hours the mixture is centrifuged. If the supernatant fluid still contains agglutinins, more culture is added and the mixture treated as before. If the agglutinating strength of the serum has not been lowered below that of the first absorption by the second addition of culture, it is certain that no further absorption by that culture is possible. If a culture is used which is identical with that used in immunization, all agglutinins will be absorbed if sufficient bacteria are added.

When the agglutinating strength is high the organisms may be removed by passage of the fluid through a Berkefeld filter. Here it must be remembered that the filter holds back most of the agglutinin until a number of c.c. have passed, and under certain conditions, as shown by Dunham, the close packing of the bacteria against the filter may continue to hold back agglutinin.

As a general rule it can be said that the agglutinins produced in an animal through the injection of any one variety of bacteria can be exhausted from the serum only by saturating it with sufficient quantities of that variety. In our experience not only the specific, but usually the common, agglutinins stimulated by it will thus be absorbed. All other varieties of bacteria will simply absorb any of the common agglutinins for which they have an affinity. If a serum is freed of all common agglutinins, it will clump only the variety of bacteria which was injected in the animal. It is practically impossible to remove absolutely all group agglutinins, since we only know that those having an affinity for the bacteria added have been removed.

The observations of Posselt and v. Sagasser,[1] that by the injections of a pure culture of one variety, agglutinins may be stimulated in large amount for other bacteria, which, however, cannot be absorbed by the variety used in immunization, have not been duplicated by us. The agglutinins not absorbed have been those present in the animal before immunization was begun, or those stimulated by the absorption of other substances. These agglutinins are abundant in horses and goats, especially for the typhoid-colon group. An agglutination of dysentery, paradysentery, and colon bacilli in dilutions of 1:1,000 has been met with by us. Considering our experience, we believe that the absorption test gives most valuable evidence, and much more than does the quantitative test, as to the identity or lack of identity between the bacterium used in immunization and the others tested against its specific serum.

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[1] *Wien. klin. Wchnschr.*, 1903, 16, p. 691.

A difficulty frequently met with among recently isolated cultures is their lack of sensitiveness to agglutinins. This is probably due to their growth in blood or in fluid which has been derived from the blood. It is known that the growing of bacteria in a specific serum, and to some extent in any serum, lessens their agglutinability. Thus we cultivated the maltose-fermenting paradysentery bacillus (Flexner, Manila), on each of 11 consecutive days, in fresh broth solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The solutions used were 1½, 4, and 15 per cent. The serum agglutinated the culture before its treatment in dilutions up to 1:800. After the 11 transfers, the culture grown in the 15 per cent solution ceased to be agglutinated by the serum, and ceased to absorb its specific agglutinins. The cultures grown in the 1½ and 4 per cent solutions agglutinated well in dilutions up to 60 and 100, and continued to absorb agglutinins. The recovery of the capacity to be agglutinated was very slow when the culture was from time to time transplanted on nutrient agar.

It seems that, growing in serum dilutions, the bacteria which developed the least agglutinable substance were least hindered in their growth, and so developed most rapidly. Those producing the least agglutinable substance were thus finally the only ones surviving.

It is sometimes difficult to tell whether a culture is non-agglutinable or simply does not agglutinate in the serum used. An absolute test is to immunize an animal with it and see if it agglutinates in the serum.

THE DEGREE TO WHICH IT IS POSSIBLE TO DETECT THE MICRO-ORGANISM EXCITING A DISEASE BY THE SERUM REACTION OF THE BLOOD OF THE INFECTED PERSON.

The success of the Gruber-Widal test in suspected typhoid fever, cholera, and a few other diseases has given most persons an exaggerated opinion of the diagnostic value of a serum reaction. Even in these diseases the information given by the serum test is not so specific as is thought by many. The serum from typhoid patients occasionally agglutinates one of the varieties of the paratyphoid bacilli in higher dilutions than the typhoid bacilli. In 30 cases tested by us this happened in two instances. Grünberg and Rolly[1] report the remarkable finding, in 40 cases of typhoid fever in which the typhoid bacilli were obtained, that in 35 per cent of the cases, the serum agglutinated a paratyphoid bacillus in higher dilutions than the typhoid bacillus. In these cases it is probably group-agglutinins, excited by the products of certain colon bacilli secondarily infecting the Peyer's patches, which agglutinated the paratyphoids, rather than the group agglutinins due to the typhoid bacilli.

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[1] *Münch. med. Wchnschr.*, 1905, 52, p. 105.

As the clinician, when considering a case of continued fever, is as a rule trying to settle whether it is one of tuberculosis, malaria, or typhoid fever, he is satisfied to know whether the infection is or is not due to one of the typhoid-colon group, and does not mind the impossibility of an absolute identification of the variety. In the case of dysentery, a quantitative agglutination test is frequently useless as an indication whether the dysentery or paradysentery bacilli are exciting the disease. In cases due to the Shiga bacillus, the serum occasionally agglutinates one of the mannite-fermenting dysentery types in higher dilutions than itself. This again is probably due to group agglutinins produced by the absorption of substances contained in certain varieties of colon bacilli. A goat injected by us with a colon bacillus produced a serum which agglutinated it in a 1:5,000 dilution, and agglutinated the paradysentery bacilli in a dilution as high as 1:2,500. Agglutination of the Shiga bacilli by a serum which does not agglutinate the paradysentery bacilli usually indicates infection with the former variety, but an agglutination of the paradysentery bacilli alone may indicate a colon infection. Different members of groups of bacteria, like the colon group or the pneumococci, though having common pathogenic properties, frequently differ almost absolutely in their reaction to agglutinins. Thus, a sheep injected by us with a typical pneumococcus agglutinated that organism in a 1:100 dilution but did not agglutinate 20 other pneumococci in dilutions higher than 1:2. An equal variation was found by us to exist among the members of the colon group of bacilli. In infections which may be due to any one of a number of varieties differing in their agglutination characteristics, it is almost impossible to use sufficient cultures to diagnose by the serum reaction whether one of the group was the exciting factor.

The greatest limitation to the use of the serum reaction is the fact that the majority of bacteria do not, in the course of an infection, excite a sufficient amount of agglutinin to be readily detected, as for instance in the case of tubercle, influenza, and diphtheria bacilli.

Bacteria widely separated may, in exciting great quantities of agglutinin for themselves, develop so much group agglutinin for each other as to be misleading. An animal injected with staphylococcus agglutinated the typhoid bacillus in 1:160, while before, only 1:10. Another, injected with B. proteus, agglutinated a culture of this in 1:160,000, and also the typhoid bacillus in 1:1,200. In such a case, if the typhoid bacillus was suspected as the cause of the infection as above tested, the serum reaction would be apt to deceive.

In actual natural infections such very high reactions are improbable, but those sufficiently high to give misleading group reactions frequently occur. In adults the blood is apt to contain a considerable amount of group agglutinins for many bacteria before the special infection which is to be investigated developed. It is only through long experience that we are able to determine in how high dilutions such agglutinins are apt to act, and therefore in what dilutions a specific reaction can be suspected or considered proven. In suspected typhoid infection, for instance, we are now able to state that a reaction in a 1:50 dilution in two hours at room temperature is proof of an infection with a member of the typhoid-colon group, and as the great majority of such infections are due to the typhoid bacillus, we can consider this as the probable microorganism. Agglutination of the typhoid bacillus in higher dilutions makes this probability almost, but not quite, a certainty.

COMPARATIVE STUDIES OF DIPLOCOCCI DECOLORIZED BY GRAM'S METHOD, OBTAINED FROM THE SPINAL FLUID AND FROM THE NARES OF CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS.

Edward K. Dunham.

The studies which are here epitomized were undertaken as a part of the work of the Commission for the Study of Cerebrospinal Meningitis appointed by the Department of Health of the City of New York in March, 1905. The Commission deemed it important to ascertain whether microorganisms identical with those occurring in the cerebrospinal cavity were to be found in the upper air passages of those suffering from epidemic meningitis; and a part of the cultural work for the Commission was the isolation, from the nares of such cases, of diplococci not readily distinguishable from the diplococcus of meningitis by morphological characters.

Pure cultures of the Diplococcus intracellularis (Weichselbaum) were secured from the spinal fluid obtained by lumbar puncture from 50 cases of epidemic cerebrospinal meningitis and served for comparison with the cultures from the nares.

CULTURES.

The organisms from the nose or throat were obtained from streak-plate cultures made from the secretions collected with sterile cotton swabs. The medium employed for these plate cultures was 2 per cent glucose agar, enriched with ascitic fluid or sheep serum mixed with the melted agar at the time the plates were poured, or by distributing a little human or rabbit blood upon the surface of the agar after it had solidified. Cultures from the spinal fluid were obtained upon similar agar plates. Subcultures were made upon slants of 2 per cent glucose agar with or without other addition, or with a little blood or sheep serum upon the surface. The cultures on 2 per cent glucose agar without enrichment have usually exhibited excellent growth, provided the medium was protected from drying. Even the slight drying due to loose cotton plugs has greatly impaired the growth; but when the plugs were tight, so that the surface of the medium was kept in a moist atmosphere, abundant growth could almost always be secured, and the cultures remained alive for a comparatively long time. To this end the air in the thermostat was also kept moist by placing a dish of water on the lowest shelf.

The cultures from spinal fluids upon 2 per cent glucose agar are gray and moist, usually only slightly raised above the surface of the medium, and appear to be strictly confined to those portions of the medium that were directly inoculated, there being no tendency to spread upon its surface. The condensation water becomes cloudy, with the formation of an abundant sediment, and, frequently, a slight pellicle is perceptible upon the surface of the condensation water. In cultures that are three or four days old the growth has a slight mucinous consistency, but is easily removed from the surface of the agar with a platinum loop. When scraped from the agar, the mass of bacteria has a pinkish buff color, and is readily broken up and disseminated in water or saline solution. A considerable variation in the luxuriance of growth was noted in many of the subcultures from different spinal fluids. In some cases a much more abundant inoculation of fresh media was necessary for success than in others, and the resulting growth was in the form of discrete colonies rather than a uniform layer. In other cases the growth was so abundant and formed such a thick layer upon the media as to excite the suspicion that the cultures were contaminated. These variations in growth depend not merely upon the particular strains under observation, but also upon the media employed. Slight indeterminate variations in the latter affected the luxuriance of growth very markedly. In two or three instances a particular lot of glucose agar was found to be unfit for use, even an abundant inoculation yielding no growth, although the preparation of the media could not have differed greatly from that employed when the result was most favorable.

An explanation of these experiences cannot be given; the method of preparing the medium had become an established routine from which there were not conscious departures.

Owing to the variations noted in the cultures on 2 per cent glucose agar, it is impossible to formulate a typical description of the growth of the D. intracellularis on this medium that would serve for its certain identification.

Notwithstanding this fact, the cultures of diplococci decolorized by Gram's method of staining and obtained from the upper air passages could, in many instances, be readily distinguished from D. intracellularis when grown on 2 per cent glucose agar. These easily recognized as differing from this diplococcus were divisible into three groups:

1. Those yielding very luxuriant, heavy, mucinous growths of a gray color and forming a much thicker layer upon the agar than those of D. intracellularis. These cultures readily disseminated in saline solution and yielded a good suspension upon filtration through paper. They failed, however, to agglutinate in very low dilutions (1:20 or 1:50) of serum from a horse immunized with D. intracellularis from spinal fluids, while the latter was agglutinated in dilutions of 1:500 to 1:1000.

2. Those giving rise to very coherent growths that adhered strongly to the surface of the agar, so that it was extremely difficult to remove any considerable quantity of the growth with a platinum loop. This growth did not disseminate easily in saline solutions and was almost entirely removed by filtration through filter paper, so that satisfactory suspensions could not be obtained for agglutination tests.

3. Cultures which, within one to three days, produced a greenish-yellow fluorescence in the agar.

After eliminating these three groups, there remained:

4. A group of cultures which could not be readily distinguished by the gross appearances of the growth on 2 per cent glucose agar from cultures of D. intracellularis on the same medium. This group was of greater interest in the present inquiry than the other three and received most study. It could be divided into two subgroups: those more closely resembling Micrococcus catarrhalis, and those more closely resembling, if not identical with, D. intracellularis. These two subgroups may be provisionally designated as catarrhalis-like and intracellularis-like groups. The former were certainly not all of the same species, and it is doubtful whether any of them were identical with M. catarrhalis of Ghon and Pfeiffer. The intracellularis-

like group of diplococci appeared to be quite homogeneous, and there is reason to believe that they were identical with D. intracellularis of Weichselbaum. Coverglass preparations of the catarrhalis-like diplococci show somewhat less variations in size, form, and intensity of stain than similar preparations of D. intracellularis from spinal fluids, but these differences do not appear marked enough to inspire confidence in them as a means of distinguishing with certainty these diplococci from D. intracellularis. There was, however, a difference in the suspensions in 0.75 per cent solutions of salt. The organisms from the cultures of catarrhalis-like cocci showed a distinct tendency to settle and form a sediment within a few hours (although they readily passed through filter paper), while those of the intracellularis-like cocci remained in suspension for a very much longer time. This difference was constant. Within 24 hours a suspension of the growth from an agar culture of the former, one day old and containing enough organisms to render it distinctly opalescent, would yield a marked sediment when kept at rest over night in one of the tubes used for macroscopic agglutinations. Suspensions of D. intracellularis of like density failed to exhibit such sedimentation. In one instance such a suspension was kept in the ice-box for three weeks without more than a trifling sedimentation. These differences rendered macroscopic agglutinations of the catarrhalis-like group, for purposes of comparison, extremely difficult. Further differences, revealed by the readiness with which sera containing catarrhalis-like or intracellularis cocci could be filtered through Berkefeld filters, will be referred to later.

AGGLUTINATIONS.

Comparison of the various cultures of the fourth group and D. intracellularis was attempted with the use of macroscopic agglutination tests. For each test 1 c.c. of the serum properly diluted was mixed with 1 c.c. of a suspension of the organism in 0.75 per cent salt solution. In nearly all of these studies the final dilutions of the sera in each series of tests were: 1:20, 1:50, 1:100, 1:200, 1:1000, and 1:2,000. In a few cases higher dilutions were made, but did not add to the value of the results. The mixtures of serum, saline solution, and bacterial suspensions were placed in the incubator (37°) for three hours and then in the ice-box (about 5°) over night. At the end of three or even six hours there was usually no well-marked evidence of agglutination, but after 24 hours the lowest dilutions showed distinct clumping. The first routine observations were made after from 15 to 24 hours, and a second observation from 42 to 48 hours after the tests were placed in the incubator. In the majority of instances the second observation revealed agglutination in one or two of the dilutions next higher than those giving evidence of this reaction at the first observation. The time which was allowed to elapse before making the observations is much longer than the usual limit adhered to in making macroscopical tests on agglutination. But it is believed that no errors due to growth of the organisms could vitiate the results. It is certain, from experiments made to determine this fact, that much more rapid reactions might have been secured if broth cultures of D. intracellularis had been used instead of suspensions from agar cultures. The saline suspensions were preferred, because it was believed that more uniform results could in this way be obtained in making tests at different times with an organism so capricious in vigor of growth as is intracellularis Suspensions of apparently identical richness could be prepared without great difficulty at any time. The delay in reaction appeared to be relatively unimportant. In some of the tests $\frac{1}{4}$ per cent of carbolic acid was added to the salt solution to preclude growth of the organisms. This addition did not in any way modify the results.

The sera employed in these agglutination tests were either from patients suffering from epidemic cerebrospinal meningitis or convalescent from this disease, or from animals which had been immunized with either single or repeated injections of large quantities of D. intracellularis obtained from spinal fluids. The animals used were rabbits, a goat, a horse, and several geese. The animals usually bore the injections of cultures from spinal fluids well. The most susceptible were the rabbits. But these soon acquired a considerable tolerance for even very large quantities, manifesting very slight, if any, acute symptoms, though they gradually became greatly emaciated, and lost much hair when the injections were continued for a long time. The geese were very tolerant of D. intracellularis. In one case about 5 c.c. of a thick purée prepared from 14 plate cultures (each about 10 inches in diameter) incubated for 24 hours, was injected into the pectoral muscles of a goose without marked effect either locally or in the general condition. The animal refused food for a few hours, but on the next day ate as usual. The cultures of catarrhalis-like cocci and other diplococci, certainly not meningococci, from the nose and throat were often much more virulent for geese and rabbits when given subcutaneously or intraperitoneally, the first injection frequently causing death.

The goat was immunized by Dr. Simon Flexner, who kindly furnished the serum for these tests. Large quantities of D. intracellularis from the spinal fluids obtained from 13 cases of epidemic meningitis were used by him to immunize this animal, and the injections were repeatedly given during a period of many weeks.

The horse serum came from an animal immunized with large quantities of dead cultures at the Research Laboratory of the New York Department of Health, and was generously contributed by Dr. Park. Some of the sera were preserved with chloroform, trikresol, toluol, or $\frac{1}{4}$ per cent phenol; others received no preservative, but were collected and kept uncontaminated. Comparative tests failed to reveal any influence exerted by these additions upon the agglutinating power of the sera.

None of these sera showed a high specificity in their agglutinations of D. intracellularis. They agglutinated in dilutions from 1:200 to 1:4,000, or two to ten times as great as the sera from normal animals of the same species. But it is difficult to utilize even this moderate increase in agglutinating power, because the conditions under which the tests were made differed unavoidably at different times, and even slight variations in condition influence the limits of the dilution at which positive results are obtained in a very great degree. The density of the emulsion, for example, was found to make a very great difference in the development of distinct agglutination in the higher dilutions. This fact is probably related to the observation that a complete agglutination of all the organisms in an emulsion, with a sedimentation of the resulting clumps, rarely takes place when the macroscopic method and saline suspensions are used. Free organisms are almost always present in sufficient abundance to render the fluid perceptibly opalescent, even when the agglutination of the remainder of the organisms gives rise to relatively large and heavy clumps that settle to the bottom of the liquid. Complete agglutinations are, therefore, comparatively rare when this method is employed. They appear to be much more common, especially in the lower dilutions, when broth cultures are used in place of suspensions in normal saline solution.

The fact, already mentioned, that the suspensions of catarrhalis-like diplococci tended to form a distinct sediment within 24 hours, rendered a comparison of the agglutinating power of the sera upon this group of organisms with the agglutination of intracellularis cocci practically impossible, but this sedimentation itself furnished a means of distinguishing these cultures from those of the latter organism. The difference between an agglutination with settling of the clumps and a sedimentation of the individual organisms is readily demonstrable upon moderate amplification. For this purpose a microscope with the tube in a horizontal position and a Leitz No. 3 objective were used to control the macroscopic observations. By rotating and then suddenly tilting the agglutination tube the sediment could be thrown up from the bottom along the side of the tube and readily examined with the microscope. The latter was placed in front of a window and the mirror removed. A very small diaphragm was employed, and the individual bacteria could be distinguished without difficulty. This microscopical control, therefore, served also to eliminate contamination with organisms morphologically differing in marked degree from D. intracellularis which might occasion errors in the observations.

The foregoing observations suffice to show the futility of an attempt to use these methods of agglutination in the comparative study of *all* the diplococci decolorized by Gram's stain found in the nose and throat, and those obtained from spinal fluids. In some cases they simply demonstrate constant differences in the suspensions of different cultures.

It was thought that more obviously biological differences or likenesses of general applicability might be revealed if the various agglutinins in the sera were removed by absorption with large quantities of a given species, and the serum thus deprived of one set of agglu tinins applied to suspensions of the various organisms under study, with a view to determining which of these species were then agglutinated. Many such experiments were made, with apparently gratifying success, but a critical review of the results casts much doubt upon their significance and also reveals unexpected difficulties in technique, which have not yet been overcome.

It was found that D. intracellularis from spinal fluids apparently removed all the agglutinins capable of clumping the organisms derived from the spinal fluids and also, as far as could be determined, those agglutinating the catarrhalis-like organism and other members of the fourth group from the nares; whereas the latter failed to remove from the serum of animals immunized with D. intracellularis more than a fraction of the agglutinins clumping D. intracellularis. But the very heavy suspensions necessarily used to insure absorption of the agglutinins were very different in the two cases. It was comparatively easy to separate the catarrhalis-like organisms from the diluted serum (usually diluted 1 in 5 or 1 in 10 with normal salt solution) either by centrifugalizing or by filtration through a Berkefeld filter. But it was found to be impossible to free the serum sufficiently from D. intracellularis with any available centrifuge. Although most of the organisms were thrown down, so large a number remained in suspension that the use of the serum was very unsatisfactory, especially in view of the delayed reactions already described. The separation of the serum by filtration through a Berkefeld filter, while successful, was exceedingly slow in comparison with the similar filtration when cultures of the catarrhalis-like cocci were employed. The heavy suspensions of D. intracellularis quickly formed a dense coating upon the surface of the filter, while an equally dense suspension of the catarrhalis-like organisms was more granular, or less slimy, and the deposit upon the filter impeded the passage of the serum in a very much less degree. This difference in the physical character of the two suspensions, which persisted in considerable, though less degree, even when the centrifuge was used before filtration, excited the suspicion that the agglutinins of the serum were mechanically held back by the coating of organisms or slimy substances in the suspension, and this suspicion was increased by the observation that a heavy suspension in diluted immune serum of a culture from the throat, which was certainly neither D. intracellularis nor one of the catarrhalis-like group, but which produced much mucinous material on 2 per cent glucose agar, also filtered exceedingly slowly and removed nearly if not quite all the agglutinins clumping D. intracellularis and intracellularis-like diplococci. If the loss of agglutinins were due to a mechanical prevention of their passage through the clogged filter and not to a union with the organisms, the value of these absorption tests, when all the agglutinins have been removed, is so seriously impaired as to cast doubt on their having any specific value, though they do reveal differences in the cultures used for absorption and, for this reason, are not without significance.

To test the effect of clogging a Berkefeld filter with a colloid so as to lengthen the time required for the passage of a diluted serum, a 1 per cent colloidal solution of corn starch boiled in 0.75 per cent salt solution was used as a diluent of the immune serum, and compared with a similar dilution with normal salt solution used as a control. The latter passed through a Berkefeld filter in 15 minutes. The starch mixture required about 20 hours, or 80 times as long. The agglutinating limits of the starch filtrate upon various of the organisms under study was found to be only $\frac{1}{4}$ to $\frac{1}{10}$ that of the control. This result appears to support the idea that the agglutinins might be mechanically held back by the coating upon the filter when intracellularis was used for absorption. It is possible, however, that the colloidal starch may have had some direct action upon the agglutinins in the serum. But this might also be the case with collodial (slimy) substances in the cultures of D. intracellularis. That the agglutinins may in this case be mechanically separated by filtration is also suggested by an observation indicating that the removal of the major part of the diplococci from the diluted serum with the centrifuge somewhat hastens subsequent filtration, but furnishes a filtrate with greater agglutinating power than it possessed when filtration was slower.

Notwithstanding the fact that these absorption tests have failed to establish a definite union between specific agglutinins in the immune sera and the intracellularis organisms, they throw some light on the relations between D. intracellularis and the diplococci of the fourth group found in the nose and throat. Some of the latter were identical in behavior with D. intracellularis; others yielded suspensions much more readily filtered or freed from the organisms with the centrifuge.

FERMENTATION.

A more striking biological distinction between D. intracellularis and the catarrhalis-like group of cultures was the differences they exhibited in the production of acid when grown in a suitable medium containing dextrose. Twenty-six of the cultures from the spinal fluid, three from the blood, and sixteen from the nose or throat of patients with epidemic meningitis were studied with a view to determining the production of acid when grown in the presence of dextrose.

The medium employed was a mixture of nutrient broth containing 1 per cent dextrose, sheep serum diluted with three times its volume of water, and 1 per cent solution of Kahlbaum's or Merck's purified litmus. These three constituents were separately sterilized in the Arnold sterilizer on three successive days, and then one part of the diluted serum mixed with three parts of the broth and enough of the litmus solution added to impart a distinct color to the whole. The mixture thus prepared was incubated for two to three days to detect accidental contamination. All of the cultures of D. intracellularis from spinal fluids and all of the cultures from the blood produced an unmistakable acid reaction in this medium after incubation at 37° for 24 hours. The intensity of this reaction usually increased slightly during the next 24 to 48 hours, but in no case was the acid production sufficiently abundant to occasion coagulation in the medium. When the reaction has reached a certain degree of acidity it appears to remain about constant, and to render the medium unfavorable for the further growth of the organisms. At the end of nine days only three out of 29 such cultures were found to contain living organisms. Similar cultures made with the organisms from the nose and throat differentiated them into three groups: (1) those which produced acidity without coagulation, i. e., the same change wrought by D. intracellularis; (2) those causing the formation of acid with coagulation in 24 hours; and (3) those producing no acidity (or, perhaps causing an alkaline change of reaction) even after nine days' incubation.

Of the 16 cultures from the nose and throat, six affected this broth-serum-glucose medium in exactly the same way as D. intracellularis. Of these six cultures a few had already been used for observations concerning their susceptibility to agglutination and their ability to absorb agglutinins from immune sera. In all these respects and in the characters of the suspensions they furnished, and in their various cultures, they corresponded to parallel observations on D. intracellularis. As far as our knowledge can justify a conclusion, these cultures were identical with this diplococcus and the latter were, therefore, present in the upper air passages in the cases of epidemic meningitis from which these cultures were obtained.

Most of the work furnishing the results here summarized was done during the summer of 1905 with the assistance of Mr. Hubert C. Ward, to whom grateful acknowledgment for many helpful suggestions and unflagging interest is cordially extended.

THE FREQUENT OCCURRENCE OF MENINGOCOCCI IN THE NASAL CAVITIES OF MENINGITIS PATIENTS AND OF THOSE IN DIRECT CONTACT WITH THEM.*

Mary E. Goodwin and Anna I. von Sholly,
Assistant Bacteriologists, Research Laboratory.

(From the Research Laboratory of the Department of Health, Dr. William H. Park, Director.)

Everyone familiar with the investigations concerning the etiology of meningitis knows that, owing partly to the difficulty of isolating and keeping alive the meningococcus, partly to its similarity to other micrococci, the work of most investigators has been incomplete and therefore of little permanent value.

As the amount of influence which the results of the investigations here recorded may exert depends largely on the degree to which others are convinced of the thoroughness of the identification of the organisms found in the nasal cavities, it seems best to review briefly the literature in order to see what characteristics the best observers consider as belonging to the meningococcus and therefore as necessary to prove the identity of the suspected organism.

The first important study of the etiology of primary cerebro-spinal meningitis was undertaken by Weichselbaum in 1887. Before that time it had been pretty well established that in secondary cases the pneumococcus was at times the exciting factor, though Leyden[1] and Leichtenstern[2] had noted diplococci in the exudate of fatal cases of primary cerebro-spinal meningitis which they believed to be different from pneumococci. Their descriptions lead one to think that they really saw the meningococcus, but their work was too meager to establish this.

In 1887 Weichselbaum[3] isolated, and carefully studied, cultures from six typical cases of cerebro-spinal meningitis. The cocci had the following cultural characteristics. They grew well on nutrient agar-agar containing 2 per cent of gelatin. The growth on the surface was rather flat and viscid; it was gray in direct, and grayish-white in transmitted, light. The borders were indented and showed the growth to be made up of confluent colonies. Potato showed no visible growth. On the agar-gelatin plates the deep colonies were very small. The surface colonies were grayish white. Under the microscope they were round or irregular, finely granular and their borders indented. They had a golden brown nucleus, an inner light yellow zone, and an outer one which was transparent and colorless. Weichselbaum found it necessary to transplant the cultures every two days in order to keep them alive, as they were found to die usually in from three to six days. The cocci themselves were mostly in pairs; some were single and a few in tetrads and small heaps. The single cocci were round, the pairs flattened at the apposed ends. The cocci varied greatly in size and staining, the larger forms, which stained more deeply, being sometimes twice as large as the smaller, more faintly staining ones. They were mostly intracellular in the exudate, and were found only in small numbers in the tissues. All the cultures were Gram negative, and grew well only at blood heat. They did not grow at all at 20° C.

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* Technical portion of Part I of an investigation of cerebro-spinal meningitis carried on under the auspices of the Special Commission of the Department of Health of New York City.

In 1895 H. Jaeger[4] published the results of the study of 14 cultures isolated from typical epidemic cerebro-spinal meningitis. The organisms he isolated differed from those of Weichselbaum and more recent investigators in the following characteristics. There were short chains of four to six elements present in all the cultures, and in two cultures there were long chains of 20 to 30. He describes the cultures as being sometimes Gram positive and sometimes negative. However, he never found Gram positive cocci in the tissues. His cultures grew at lower temperature. The viability of his cultures was much greater, one culture in broth living 43 days. The culture stood drying for 96 days and pus dried on linen gave a growth of the cocci after 127 days. A capsule was present in the smears.

A. Heubner,[5] Jaeger's strongest supporter, describes cultures from four cases which were identical with Jaeger's. Jaeger in his article of 1899[6] still clings to his description of 1895. In 1901[7] he decides that the meningococcus has no capsule, but in other points holds to his original position; nor does he add anything new in his paper of 1903.[12]

Between Jaeger's first and last papers a series of investigations had been carried on which demonstrated to most bacteriologists that either he had failed to isolate the true organisms exciting the disease, or had allowed contaminating or associated bacteria to overgrow and displace the meningococci in his cultures.

Councilman, Mallory, and Wright,[8] after a thorough study of 31 cases, describe their cultures as being similar to those of Weichselbaum.

In 1901 Albrecht and Ghon,[9] after working with 22 cultures, agreed with Weichselbaum. The greater number of cultures observed led them to give wider limits of temperature as suitable for development. Some cultures grew from 25°–40°, though the maximum growth was always between 36°–37°. They are the first to describe the "bread crumb" granules found in the center of the colony after 48 hours. They give the best media as Loeffler's blood serum, or agar containing ascitic fluid. A pellicle on the broth cultures, when the broth was neutral and the cultures were left quiet for several days, was almost constant. In a few instances they kept cultures alive, when protected from drying, for 185 days without transplanting. All the cultures were Gram negative and there was no tendency to chain formation. Albrecht and Ghon obtained cultures from Jaeger and from Heubner and found them not only quite different from theirs, but also unlike each other.

Albrecht and Ghon,[10] and Weichselbaum,[11] in convincing articles published in 1903, take up the peculiarity of Jaeger's cultures point by point, and are of the opinion that he was not working with true meningococcus cultures. Taking the important points agreed upon by the best workers, Albrecht and Ghon give the following characteristics as essential in identifying true meningococcus cultures.

1. Gonococcus-like in form, dividing in the same way, always Gram negative, having many degeneration forms, and often intracellular.

2. Growing only at fairly high temperature, 25°–42°, maximum growth at 36°–37°.

3. Colonies on agar plates luxuriant, quite viscid, glistening, gray in direct light and grayish-white in transmitted light.

4. Growth confined almost entirely to surface in stab culture.

5. Develops pellicle on broth culture (when the broth is neutral and the cultures are undisturbed for several days).

6. Slight pathogenicity for ordinary animals.

7. Non-resistant.

MICROCOCCUS CATARRHALIS.

In 1901 Ghon and H. Pfeiffer[13] published the results of the study of 40 cultures of Micrococcus catarrhalis. They found that, while it grew best on blood agar, it would grow on ordinary media. It differed from the meningococcus in growing more easily, more luxuriantly, and at a lower temperature. The colonies under the microscope were darker, more compact, and had more abrupt margins. Jaeger[14] finds all the strains of M. catarrhalis self-agglutinating. Some of the cultures examined by us have had all the above characteristics, while others have more nearly resembled the meningococcus.

MENINGOCOCCUS CULTURES ISOLATED BY PREVIOUS INVESTIGATORS FROM THE NASAL MUCUS.

In going over the literature we are impressed with the small number of cases from which thoroughly identified meningococcus cultures have been isolated from the nasal mucus. The cases from which Gram negative diplococci closely resembling meningococci have been found in the smears from the nose and throat are, on the contrary, numerous, and have been found by nearly all workers on meningitis.

The first to identify as meningococcus a culture taken from the nasal mucus was F. Kiefer.[15] While working with meningitis cultures he developed a severe purulent rhinitis. The pus contained numerous meningococci. In 1898 Schiff[16] isolated cultures from three out of 29 dispensary patients, a portion of whom suffered from chronic laryngitis, which cultures, he says, Weichselbaum considered true meningococci. These three cultures will be considered later in connection with two obtained by us from medical students, which agreed with the meningococci obtained from the spinal fluid in all respects except in agglutination characteristics. Councilman, Mallory, and Wright[8] report one culture from the throat of a tonsilitis case. Griffon and Gandy[17] twice, at an interval of five days, isolated cultures from the nose of a meningitis case which were identical with cultures from the spinal fluid. Albrecht and Ghon report two instances, one from a case of meningitis, the other from a man whose child died of meningitis three days before the culture was taken. F. Lord,[18] of Boston, isolated meningococci from a case of rhinitis. A. Weichselbaum and Ghon[19] identified one culture from the nose of a meningitis patient and three from the noses of people in contact with patients. These cultures from the 14 cases were the only ones we could find that were studied with sufficient care to warrant their acceptance as true meningococci.

ORIGINAL INVESTIGATION.

Most of the material for this investigation was obtained through the courtesy of Dr. A. W. Taves, of Gouverneur Hospital.

The mucus was taken from the nasal fossæ with a sterile cotton swab and plated out as soon as possible on ascitic agar. As a rule the plates were made within one hour of collecting the specimen, while the swab was still moist. These plates were incubated for from 24 to 48 hours, then fished in the usual way. The colonies were put on blood agar, which seemed to be the most favorable medium.

Several colonies were fished from every type found which resembled a meningococcus colony in color or granularity, and which, under the high power, showed diplococci resembling meningococci. The organisms from the cultures were stained by Gram, and several of the Gram negative ones, which in cultures resembled meningococci, were kept for study.

Fifty-two meningitis cases were examined. Meningococci were isolated from 12 of the 22 cases examined during the first week of the disease, and from 5 of the 15 examined during the second week. In six cases examined during the third week, three during the fourth, and six between the fifth and ninth weeks, no meningococci were found, while in a very severe case examined on the 67th day, we found a few colonies. In one case we failed to get them on the first day and found them in large numbers on the second.

From this it would seem as though the meningococci were present in a rather large percentage of the cases during the first week of the disease.

The nasal secretions of 45 healthy persons living in close contact with meningitis patients were examined. In five of these, meningococci were isolated during the first two weeks of the patients' illness. From the nasal mucus of 55 first year medical students who had never been in known contact with meningitis, there were isolated in two cases a few organisms which were, culturally and in pathogenicity, like meningococci. In studying their agglutination, however, we found that they differed from our other cultures in their specific agglutinins, and therefore were differentiated in one important respect from the latter. In this connection it is of interest that Schiff, in describing his cultures from the nasal cavity of people not in contact with meningitis, does not refer to agglutination, and evidently did not make the test. His cultures may have differed as ours do. One cannot safely classify these atypical cultures. They may be meningococci derived from a strain different from those isolated by us in the present epidemic, or organisms not capable of readily exciting meningitis, and yet so closely related that they cannot be differentiated without more careful cultural tests than we at present use.

The following tables give the cases, the day of the disease when the specimen was taken, the termination of the disease, and the bacteriological findings.

TABLE 1.

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Disease	Termination	Percentage of Meningococcus Colonies Present in Plates
W. W.	1	Died	About 55
J N.	2	Died 3d day	“ 90
L. Z.	3	?	“ 30
E. R.	3	Died 4th day	“ 50
R. T.	3	“	“ 40
J. G.	4	?	“ 95
Mrs. M.	4	Died 6th day	Very few
S. F.	5	Died	About 50
S. K.	5	?	“ 90
J. S.	5	?	“ 30
D. M.	7	Recovered	“ 2
C. P.	7	?	Few
M.	10	Died	About 10
M. G.	10	“	“ 2
J. M.	12	“	A very few
M. H.	14	?	About 95
E. S.	14	Died	“ 5
S. K.	67	Died 69th day	“ 2

TABLE 2.

CONTACTS WITH MENINGITIS CASES FROM WHOM MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Patients' Illness	Time Since Last Contact	Condition	Percentage of Meningococcus Colonies Present in Plates
Mr. D.	....	4 days	Normal	About 95
Mrs. D.	....	4 “	“	“ 95
Mrs. K.	14th day	Still in contact	“	“ 95
A. K.	14th “	“ “ “	“	“ 50
Mrs. M.	....	14 days	“	“ 30

The plate cultures from the mucus of all these cases contained many colonies and in most cases great numbers of colonies.

TABLE 3.

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE NOT ISOLATED FROM THE NASAL MUCUS.

No. Examined	Day of Disease	No. Examined	Day of Disease	No. Examined	Day of Disease
1	1	2	13	1	24
1	2	5	14	1	27
2	3	1	15	1	28
5	6	1	17	1	31
1	7	1	18	1	40
1	9	1	19	1	42
1	10	1	20	1	49
1	11	1	21	1	60

TABLE 4.

CONTACTS WITH MENINGITIS CASES FROM WHOM NO MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Number Examined	Days since Contact	Number Examined	Days since Contact
5	2	1	35
9	3	1	50
2	4	3	56
1	10	1	60
1	18	16	Still in contact

All contacts were occupants of the same rooms and nearly always members of the family.

From 14 cases we took multiple specimens. In only one case did we find meningococci in two specimens, 90 per cent on the fifth day, and a very few on the tenth. Table 5 gives the cases, the day of disease, and the bacteriological findings.

CULTURAL CHARACTERISTICS OF THE MENINGOCOCCI ISOLATED FROM THE NASAL MUCUS.

The cultures isolated from the nasal mucus were carried out on the different laboratory media and compared with 30 cultures isolated from a similar number of specimens of spinal fluid.

There were no apparent differences between the nose and spinal fluid cultures. Some grew more luxuriantly than others. The more luxuriant cultures from both spinal fluid and nose seemed to have a more yellow tone, while those growing in a thinner layer were grayish-white.

The morphology of the organisms differed slightly, but the differences were the same for cultures from both sources.

The meningococci occurred as flattened cocci in pairs, fours, and sixes. They varied widely in size in the same culture from the same media, and differed greatly in the intensity with which they took the stain.

In no case did a culture tend to be Gram positive. Cultures were repeatedly plated out, and numerous colonies fished and stained by Gram. In a culture transplanted twice a day for five days on Loeffler's blood serum, so that the organisms might all be very young; there was no tendency for any of them to be Gram positive.

TABLE 5.

CASES OF MENINGITIS FROM WHICH MULTIPLE SPECIMENS WERE EXAMINED.

Name	Day of Disease	Termination	Findings
B. I....................	13	Recovery	No meningococci
	14		" "
	15		" "
	16		" "
E. E....................	2	Died 7th day	" "
	3		" "
G. D....................	7	Died 45th day	" "
	13		" "
	19		" "
K. S....................	5	?	90 per cent
	10		A few
	16		No meningococci
M. D....................	7	Recovered	2 per cent meningococci
	13		No meningococci
	19		" "
C. P....................	3	?	" "
	4		5 per cent meningococci
	5		No meningococci
	7		" "
	8		" "
	9		" "
S. M....................	2	?	" "
	6		" "
	8		" "
S. J....................	6	Recovered	" "
	7		" "
	9		" "
	10		" "
S. T....................	3	Died 10th day	" "
	4		" "
S. J....................	5	?	30 per cent meningococci
	10		No meningococci
	18		" "
W. F....................	3		" "
	4		" "
W. J....................	17	?	" "
	18		' "
	19		" "
	20		" "
	22		" "
Z. L....................	1		" "
	3		30 per cent meningococci

The method of staining by Gram was the same throughout the work and was briefly as follows:

1. Stain two minutes in anilin gentian violet
2. Displace anilin gentian violet with Gram's Iodine Solution and leave on one and one-half minutes.
3. Wash in 95 per cent alcohol until visible color stops coming out.
4. Wash in water and counter stain 30 seconds in watery solution of Bismarck brown (2 grams in 100 c.c.)

In no culture was any tendency to chain formation observed. The cultural characteristics of colonies on ascitic agar plates were as follows:

1. *Macroscopic appearance.*—In many cultures there are two distinct zones, but this was not found constant on repeated plating. Where the colonies are in contact, they are usually divided by a distinct line. They are oval or irregular, grayish-white to yellowish-white, moist and usually viscid, flowing about the needle instead of breaking away from it when they are fished.

2. *Microscopic appearance: Low power.*—Pale amber to brown in color. From fine and evenly granular colonies to those with very coarse central granules. Margins generally rather even and often not abrupt.

3. *Microscopic appearance: High power.*—The diplococci, and occasionally the fours, show plainly. On some plates the margins are smoother and more abrupt, and the separate organisms are distinguished with difficulty.

The most constant characteristics seem to be the coarse central granules and the characteristic separate organisms at the margins when observed with high power.

Ascitic agar slants.—Grayish-white, fairly luxuriant growth, usually with discrete colonies. These colonies at times have a diameter of five millimeters at 48 hours. They are generally quite round, but vary a good deal in the waviness of their outlines. Two zones are often distinguished. In the smears from ascitic agar the organisms stain poorly and are indistinct.

Loeffler's blood serum.—The growth is heavy, moist, confluent and yellowish. The smears show the organisms distinctly, and usually of larger size than on ascitic agar.

Plain agar.—Growth scant, if any, and generally consisting of a few isolated colonies.

Glucose agar.—Growth slightly better than on plain agar.

Glycerin agar.—Same as plain agar.

Blood agar.—Growth very luxuriant, confluent, yellowish-white and extremely sticky; smears same as from Loeffler's.

Sheep serum agar.—Growth fairly luxuriant, about the same as ascitic agar.

Gelatin.—No cultures grew below 24°. At 37° C. all the cultures grew well, with the formation of a heavy pellicle. At the end of six weeks the gelatin still hardened when put in the ice box.

Hiss's inulin medium.—Rendered opaque but not coagulated.

Litmus milk.—The cultures grew only slightly and turned the milk somewhat darker than control at the end of 48 hours, but made no further change.

Marble broth.—Most of the cultures grew slightly, a few grew well, making the medium cloudy, afterward forming a pellicle and sediment. The pellicle was quite general after one week.

Plain broth.—Very few cultures grew in our broth and these only slightly. This was possibly due to an unsuitable reaction of the broth.

Dunham's peptone solution.—Growth very slight. Indol not produced.

Glucose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Lactose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Maltose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Saccharose litmus peptone sheep serum agar.—Acid not produced after 48 hours.

Mannite litmus peptone sheep serum agar.—Acid not produced after 48 hours.

Temperature.—The maximum growth was at about 37°. Nearly all the cultures grew at 30° three months after isolation; a few grew slightly at 24°.

Viability.—The cultures varied greatly in the length of time which they would live without transplanting. In order not to lose cultures we reinoculated them every five days. Many of the cultures on ascitic agar lived from 10 to 20 days without protection from drying, and some of the broth and gelatin cultures lived from five to eight weeks. After 25 cultures were kept in the ice box for five days none of them were alive. Cultures left at room temperature and in the ordinary amount of light varied greatly in their resistance. Most of them failed to grow after 48 hours.

AGGLUTINATION.

Weichselbaum and Ghon[19] and Bettencourt and França[20] found that the serum of meningitis patients agglutinated meningococci in from 1:10 to 1:100 dilutions. They found that the serum of animals immunized for a long time with meningococci agglutinated the cultures only in low dilutions, 1:100 being the highest. We tested the serum of very few patients. The highest dilution agglutinating was 1:200.

Finding it impossible to distinguish between nasal and cord cultures by morphological or cultural comparison, we have made use of a specific serum to aid in classifying the cultures from the different sources.

We inoculated two horses, two sheep, three goats, and 20 rabbits. Only two rabbits lived long enough to give a serum of sufficient agglutinating strength to help in our work. Of these two, one was inoculated with a nasal culture from a student not in contact with meningitis. This serum agglutinated its own culture and several typical meningococcus cultures completely in a dilution of 1:40. The other was inoculated with a cord culture, and agglutinated its own culture in a 1:400 dilution, and other cultures in a 1:50 or slightly higher dilutions.

One sheep, after being inoculated with rather large doses of a cord culture for over three months, gave a serum agglutinating most of the cultures completely in a 1:40 dilution. The goat sera never agglutinated above 1:20.

One horse was inoculated with a nasal culture obtained from a severe case of meningitis on the second day of the disease. The patient died on the third day. This horse died after a month's treatment, before the serum was of much value. The other horse was given a cord culture, and though he became very sick at the end of the first month, he improved when given smaller doses. At the end of four months the agglutinating strength of this serum was 1:100 for most of our cultures. It seemed better for some other cultures than for its own.

There was a great difference in the degree of agglutinability of the cultures on different days, which made it very difficult to compare the results quantitatively.

The following tables give some of the serum tests with cultures from the spinal fluid and noses of patients, and from the noses of

TABLE 6.

AGGLUTINATION OF 22 CULTURES OBTAINED FROM THE SPINAL FLUID, AND OF 21 FROM THE NASAL MUCUS BY SERUM OF SHEEP 182 AFTER ANIMAL HAD BEEN INOCULATED FOR THREE MONTHS.

	Control	1:20	1:50	1:100	1:200	1:400
33–2 nose	—	+	++	+	+	—
XI–2 nose	—	+	++	+	—	—
124–1 cord	—	+	+	±	|	—
VII–3 nose	—	+	+	|	—	—
W. P. 1 cord	—	+	+	±	—	—
108–5 cord	—	+	+	±	—	—
D. Getz cord	—	+	+	±	|	—
Wiesbard codr	—	+	+	+	—	—
100–2 cord	—	+	+	±	|	—
95–2 nose	—	±	|	—	—	—
140–2 cord	—	+	|	±	—	—
91–1 nose	—	+	+	+	|	—
114–2 nose	—	+|	±	—	—	—
152–1 cord	—	+	+|	±	|	—
M142–2 nose	—	+	±	±	±	—
Pregalia cord	—	+	+	±	|	—
140–3 cord						
Stolz–2 nose	—	+	±	|	—	—
Cohen cord	—	±	±	±	|	—
Fieland cord	—	+	+	+	|	—
Fieland nose	—	+	+	+	±	—
Goldfarb cord	—	+	+|	+	—	—
Schwartz nose	—	+	+	—	—	—
Goldfarb nose	—	+	±	±	—	—
182 cord	—	++	++	—	—	—
Merrit nose	—	+	±	|	—	—
136 cord	—	+	+	+	|	|
23–2 nose	—	++	+	—	—	—
IX–2 nose	—	+	—	—	—	—
Horrowitz cord	—	—	—	—	—	—
14 cord	—	++	—	—	—	—
105–5 nose	—	+	+	±	—	—
253–5 cord	—	+	—	—	—	—
36–8 nose	—	++	++	—	—	—
29–3 nose	—	+	+	+	±	|
85–1 nose	—	+	+	|	—	—
142–S cord	—	+	±	—	—	—
Bayridge cord	—	+	—	—	—	—
Rubin nose	—	±	+	±	|	—
Marzo nose	—	+	+|	±	|	—
Fielder cord	—	+	+	+	—	—
Gruno cord	—	++	++	+	|	—
McDonald nose	—	±	+	|	—	—

In testing the agglutinating power we used emulsions made from 24 hour sheep serum agar slants in normal salt solution. We used hanging drops, with the slides inverted until the moment of examination, to prevent mistaking mechanical grouping for agglutination. The hanging drops were usually examined after four hours and marked in the following way: — = no agglutination, | = trace, ± = marked trace + = good agglutination + | = very good agglutination, and ++ = complete agglutination.

contacts and from people not in contact. As a rule, the majority of the cultures seem to agglutinate as well as the culture with which the animal was inoculated.

We saturated the best horse serum with its own culture, with nasal cultures (*a*) from a severe meningitis case, (*b*) from a contact, (*c*) from a non-contact, and with several M. catarrhalis cultures. After allowing the mixture of serum and culture in a 1:5 dilution to stand for three hours, we filtered through a Berkefeld filter, and used the third 10 c.c. of the filtrate. All the meningococcus-like cultures seemed to remove the agglutinins for all the cultures, while the M. catarrhalis cultures only reduced them about one-third. The control filtration of the serum without exhaustion reduced the agglutinins about as much as did the M. catarrhalis cultures.

TABLE 7.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM		SERUM EXTRACTED WITH XI–2, A MENINGOCOCCUS CULTURE FROM THE NASAL MUCUS OF A MENINGITIS PATIENT			SERUM EXTRACTED WITH A M. CATARRHALIS CULTURE FROM A MENINGITIS CASE		
	1:20	1:40	1:5	1:10	1:20	1:5	1:10	1:20
Gruno cord	+	+	–	–	–	+ ǀ	+	ǀ
Fielder cord	+	+	–	–	–	+ ǀ	+	ǀ
142 S cord	+	+	–	–	–	++	±	±
XI–2 nose	+	+	–	–	–	++	ǀ	ǀ
33–2 nose	+	+ ǀ	–	–	–	++	ǀ	ǀ
36–8 nose	±	±	–	–	–	++	–	–
W. nose	+	+	+ ǀ	ǀ	ǀ	+	+	±

These sera after being extracted were in a 1:5 dilution filtered through a Berkefeld filter and the third 10 c.c. used.

TABLE 8.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM UNEXTRACTED		SERUM EXTRACTED WITH A SPINAL FLUID CULTURE		SERUM EXTRACTED WITH W. n., A CULTURE FROM A PERSON NOT IN CONTACT WITH MENINGITIS		
	1:100	1:200	1:10	1:20	1:20	1:40	1:100
Gruno cord	+	–	–	–	+	ǀ	–
Fielder cord	–	–	–	–	+	ǀ	–
142 S cord	+	–	–	–	+	ǀ	–
XI–2 nose	+	–	–	–	+	ǀ	–
33–2 nose	+	–	–	–	+	ǀ	–
36–8 nose	+	–	–	–	+	ı	–
W. nose	+	–	–	–	–	–	–

The sera, after being extracted, were centrifuged instead of filtered.

We saturated this same horse serum with a meningococcus culture, with a M. catarrhalis culture, and with "W. n." from a student, a non-contact case. Instead of filtering we centrifuged, and found our results somewhat different. The meningococcus culture took out all the agglutinins for the meningitis culture and not for the others, while the M.catarrhalis and the "W. n." left in over half the agglutinins for the meningitis cultures. The M. catarrhalis agglutinated spontaneously, but the non-contact "W. n." took out all of its own agglutinins.

PATHOGENICITY.

Weichselbaum, in 1887, with his original cultures, killed white mice with an intraperitoneal or intrathoracic inoculation of 5 c.c. of a broth dilution of an agar culture or of the water of condensation· The mice died in 36 to 48 hours, and the meningococci were found in the cavity inoculated and usually in the blood. Subcutaneous inoculations were without result. He killed guinea-pigs by inoculating them in the thoracic cavity; but the cocci were not found in the blood or spleen.

Three dogs inoculated subdurally with 1 c.c. and 1.5 c.c. of culture dilution died, one the same evening, the second on the third day, and the third on the 12th day. The first two showed a small amount of fluid blood between the dura and brain. There was a small area of punctiform hemorrhages deeper in the brain, and the membranes were markedly injected. Numerous meningococci were found. In the third dog, between the dura and the right cerebral hemisphere, there was thick red pus, and in the brain a hazel-nut sized abscess containing yellow pus. Around the abscess was a hemorrhagic area. The lateral ventricles contained a red fluid with flakes of pus. No meningococci were found.

Albrecht and Ghon inoculated a goat in the spinal canal. The animal developed symptoms of meningitis and died in five days. The cord showed no changes and meningococci were not isolated.

Our animal work was rather irregular in its results. By inoculating mice intraperitoneally with half of a 24 hour ascitic agar culture of either the cord or nose strains, we caused death in 24 to 48 hours. There was marked congestion of the abdominal viscera, and meningococci were found in the blood and peritoneal exudate.

Rabbits were very uncertain. A few died from subdural inoculation of rather large doses, but there were no typical lesions, and none of them contained meningococci in the blood or exudate.

With small puppies we obtained about the same results as Weichselbaum. When given a dose of two ascitic agar cultures in the spinal canal, the dogs usually died in 24–48 hours. They had convulsions and some rigidity of the neck. On autopsy the membranes were much injected and there were hemorrhagic areas in the cortex. Meningococci were found in these areas, in the fluid under the dura, and in the spinal fluid. As controls to our meningococcus cultures, we used M. catarrhalis cultures and two cultures corresponding culturally to meningococci, which had been isolated from the nasal mucus of normal medical students. The dogs inoculated with two ascitic agar cultures of M. catarrhalis did not die, while those which received the cultures from the students died in 24 hours, and gave the same autopsy results as the dogs inoculated with meningococci.

CONCLUSIONS.

Meningococci were isolated from the nasal mucus of 50 per cent of meningitis patients during the first two weeks of the disease, and from about 10 per cent of the people most closely in contact with them. They were frequently present in enormous numbers.

The two cultures isolated from normal students were like meningococci culturally and in their pathogenicity, but did not have the same specific agglutinins.

The finding of meningococci in great numbers in the nasal mucus of such a large proportion of the patients and of those caring for them, and the absence of meningococci from the nasal mucus of a large number of normal persons examined, would strongly indicate the necessity of isolating cases of epidemic cerebro-spinal meningitis, at least during the early weeks of the disease.

We wish to thank Dr. Park for his constant oversight and direction of our work.

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TEMPORARY ALTERATION OF CHARACTER OF AN ORGANISM BELONGING TO THE COLON GROUP.

OSKAR KLOTZ,
Late Governor's Fellow in Pathology, McGill University, Montreal.

IN the spring of 1904 I was given the opportunity, through the kindness of my chief, Professor Adami, to study an organism isolated by him from the water of the St. Lawrence River. The results of these studies were published in the *Journal of Medical Research*,[1] and several interesting points in regard to the interagglutination of the Coli-Typhoid group were noted.

However, aside from the agglutination phenomenon, a peculiarity in the cultural characteristics was also observed. The microorganism on solid media resembled very much the appearance of colonies of B. coli. Grown on broth the microbe gave a stringy deposit, difficult to break up on shaking, and becoming more stringy on longer incubation. In litmus milk there was a primary acidity with a subsequent alkaline reaction of the medium, but no coagulation of the milk occurred. Indol was produced only after some weeks' incubation in Dunham's broth; and of the sugar broths, gas was produced most abundantly in the glucose medium. As was noted in my publication, the organism did not ferment lactose or saccharose when first isolated from the water, but did so after it had remained on the medium for some time. The saccharose broth was found to be more easily decomposed than the lactose, the latter medium showing only a very little gas formation after several days' incubation.

After the organism had been cultivated on artificial media for some months, the following experiment was reported:

> A lethal dose of the bacillus was inoculated into the peritoneal cavity of a rabbit, and after its death (which resulted in three days), cultures were again obtained from it. The appearance of the organisms and the cultural characteristics were those of the bacilli inoculated, except that in the fermentation tubes there was a slight development of gas in the glucose broth, none in the lactose or saccharose. Transfers were made from these tubes into the respective sugar broths, that is, the glucose colony was transferred to glucose broth, the lactose colony to lactose broth, and the saccharose colony to the saccharose broth. After 24 hours' incubation there was an increased amount

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[1] *Jour. Med. Res.*, 1904, 6, p. 475.

of fermentation in the glucose transfer, but still none in the lactose or saccharose. A second transfer was made, similar to the above, and now at the end of another 24 hours the glucose and saccharose broths were both fermented; no gas appeared, however, in the lactose. In both the glucose and saccharose there was also acid production. In the lactose broth no change was evident, though there was growth in the closed arm of the tube. Four days' incubation and transfer on lactose broth gave a small amount of gas formation, and transfers from this again into lactose led to its fermentation in 24 hours. The stock culture as control produced gas in all these sugar broths in 24 hours.

This feature of the organism, its variability in the power to break up certain sugars, presented two very interesting problems. First, are we justified in making an indefinite number of varieties of B. coli, depending on cultural characteristics which may be modified artificially; and secondly, in isolating from water an organism which in the first transfers does not ferment one or more definite sugars, but which, after remaining on artificial media for some time, acquires the property, can we conclude that the microorganism has recently had an animal host?

We have repeated our experiment of passing our microorganism. the Bacillus perturbans, through an animal. In this instance we made use of the celloidin capsules as devised by McCrae,[1] which we filled with a broth culture of the bacillus.

In the table it will be noted that two sets of transfers were carried forward, the one in which the parent culture was kept on sugar-free agar, and the other in which the parent stock was on a sugar medium similar to the transfer.

The sealed capsule was inserted, aseptically, into the abdominal cavity of a rabbit on September 19, 1904. The capsule was allowed to remain in the rabbit till February 10, 1905, in all 144 days. The capsule was then again obtained, and dropped for a moment without breaking into 10 per cent carbolic acid, after which it was placed in a flask of broth (without breaking), and incubated to insure against the chance of contamination. As no growth resulted, the capsule was ruptured and the microorganism was allowed to grow in weak broth for 18 hours. Transfers were then made into the various media, including sugar broths. The organisms resembled the original bacilli of the capsule in all the media except the sugars, though the growth was not in any case so luxuriant as transfers from the stock culture.

Transfers were made from and into the respective sugar broths daily. The glucose transfer showed a very slight gas formation on the first day, and this increased from day to day for several days. The first appearance of fermentation appeared in the saccharose on the fourth day, when it was only slight, and slowly increased with succeeding transfers. However, the lactose medium offered the greatest difficulty of fermentation, as can be readily appreciated from the following table.

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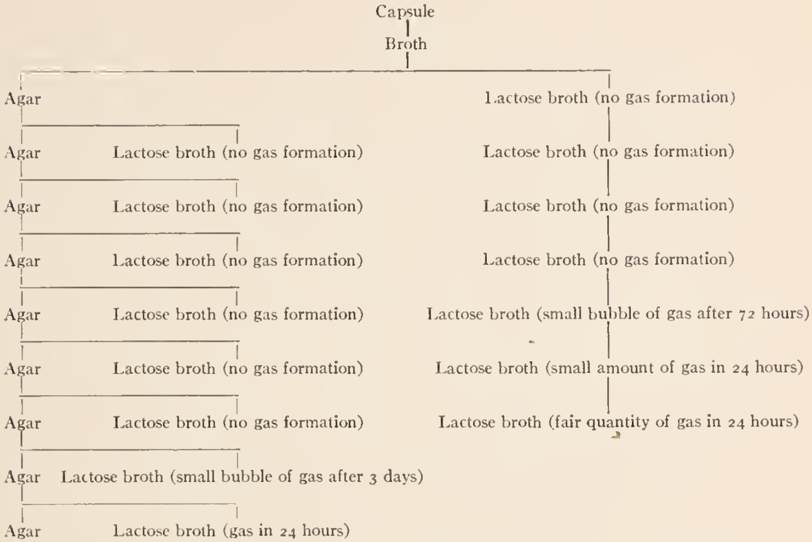
[1] *Jour. Exp. Med.*, 1901, 5. p. 635.


Capsule
Broth
Agar
Lactose broth (no gas formation)
Agar
Lactose broth (no gas formation)
Lactose broth (no gas formation)
Agar
Lactose broth (no gas formation)
Lactose broth (no gas formation)
Agar
Lactose broth (no gas formation)
Lactose broth (no gas formation)
Agar
Lactose broth (no gas formation)
Lactose broth (small bubble of gas after 72 hours)
Agar
Lactose broth (no gas formation)
Lactose broth (small amount of gas in 24 hours)
Agar
Lactose broth (no gas formation)
Lactose broth (fair quantity of gas in 24 hours)
Agar
Lactose broth (small bubble of gas after 3 days)
Agar
Lactose broth (gas in 24 hours)

As is seen in the above table, when the organism was transferred from a lactose to a lactose medium it regained its power to ferment lactose more rapidly than did the agar colonies. Having once acquired this property, the bacillus retained the lactose-splitting power in the successive transfers. In other words, the microorganism, having been deprived of one of its functions of altering the composition of certain materials by forced growth or environment, may again regain this function if it remain in contact with the material over an extended period.

Peckham[1] has given us the most complete study of the influence which environment exerts on the characters of organisms, especially of the colon group. In some cases this alteration consisted in an excessive activity of one function, in others the opposite, certain traits of the bacillus being entirely lost. In a series of cultural experiments, she was able to force B. typhosus to produce indol.

Of the external influences which can be brought to bear on bacteria, alteration of the quantity or quality of the food supply plays the most important rôle, and leads to modification of their biological nature. Thus some bacteria, in their normal metabolism, if we may so call the cell activity, secrete enzymes which split up proteids;

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[1] *Jour. Exp. Med.*, 1897, 2, p. 540.

others secrete ferments acting on sugars. The colon bacillus, among others, possesses a proteolytic ferment, whose activity we estimate by the quantity of indol produced in the medium. If, however, the colon bacillus be grown over an extended time in river water, its power to produce indol is diminished or entirely lost; while again, as was said above, if a non-indol producing organism, such as the typhoid bacillus, be grown in a medium containing proteids alone, it acquires the property of producing indol.

Other examples of the influence of environment on bacteria are well known. Jenner[1] found that he could revert B. coli capsulatus to an unencapsulated form by cultural methods. The new variety then possessed characteristics dissimilar to the previous capsulated form; as for instance, while the capsulated bacillus coagulated milk, the unencapsulated stock lost this power when placed in this medium. A more remarkable difference was noted in the pathogenesis of these two varieties, for, as we know, B. coli capsulatus is very pathogenic for white mice, but becomes less fatal or even non-pathogenic on losing its capsule.

Experimenting with this same organism, B. coli capsulatus, Larulle[2] reports similar results of transforming his "opaque" variety into the "transparent," by passing the former through animals.

Other examples of alteration to a lesser degree in the characters of bacteria are seen in the everyday cultures, in the increase or decrease of the amount of acid produced, the morphological change which organisms undergo when inoculated on different media, and many other variations.

In a paper on the variability of bacteria Adami[3] discussed the alterations of character in bacteria due to environment, which give rise to different races of microorganisms. There he pointed out that two kinds of variations may occur, the temporary variation, in which the microorganism acquires characters that are lost after several transfers have been made, and the permanent variation, in which a new function or change is impressed on a microbe and remains with it in all future cultures. Of the latter class there are not many, for we must remember that what we call permanent is but a relative term. We speak of the characters as permanent when, after weeks, months, and years, no change is noted in the transfers from the type of the parent stock.

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[1] *Jour. Path. and Bact.*, 1898, p. 257.

[2] *La Cellule*, 1889, 5, p. 61.

[3] *Medical Chronicle*, 1892, 16, p. 366.

That at least temporary modifications can be brought about by such simple methods of cultivation and in so short a space of time seems to me to indicate that among those which we call varieties of B. coli there are some which owe their differentiating qualities to a prolonged habitat in a medium differing from that in which the parent stock has had its growth, and that through subsequent growth in suitable media the original qualities of the parent stock may be acquired. Our culture medium is at best a poor imitation of the natural habitat of these minute, and, I might say, impressionable, living bodies; hence we can conceive that investigators may obtain different results with the same organism. Thus with the colon bacillus it would seem that so long as we bring forward new sugars to ferment, we get an equal number of new varieties.

Further, when organisms, which under ordinary conditions produce gas in sugar media, are found to have lost this quality, it is one of the alternatives that the organism has been a parasite in the animal body. In our own case the same organism was also isolated from sewage flowing into the river, and the reactions of this strain of the microbe on media were the same as described, that is, it was primarily a non-lactose fermenter, but later acquired the property to break up this sugar.

THE LONGEVITY OF BACILLUS TYPHOSUS IN NATURAL WATERS AND IN SEWAGE.

H. L. Russell and C. A. Fuller.

(From the Wisconsin State Hygienic Laboratory and the Bacteriological Laboratories of the University of Wisconsin.)

INTRODUCTION.

In a paper published in this *Journal* in 1904,[1] Jordan, Russell, and Zeit detailed an extensive series of experiments on the longevity of the typhoid bacillus in water, in which simultaneous, though independent, tests were made on this organism as exposed to the waters of Lake Michigan, the Chicago River, the Chicago Drainage Canal, and the Illinois River. The methods used in this study and the results obtained were so different from those which have previously been reported that it seems desirable to test this question further, employing waters of different origin.

The attempt was made in all of this work to approximate, as closely as possible, the conditions that exist in nature, and, for this reason, a marked change in technique was instituted. Heretofore, it has been customary for experiments on the longevity of bacteria to be made in glass containers, filled with sterile or raw waters. The conclusions based on work under these conditions have been shown to be erroneous, and in the work previously referred to, the method was adopted of exposing the typhoid organism in permeable sacs (celloidin and vegetable parchment), filled with the type of water in which the sacs were suspended. If, then, any variation occurred in the composition of the stream in which the sacs were exposed, the influence of such variation, if of any effect, should be felt on the imprisoned cultures within the sac.

The results obtained in the experiments conducted on the Chicago Drainage Canal and other waters showed a marked variation in the vitality of B. typhosus. In the relatively pure waters of Lake Michigan, this organism could be recovered readily from the infected sacs, for a period of at least a week, while in the highly polluted waters of the Chicago River and the Drainage Canal, the longevity of the same strain, exposed in a similar way, was reduced to two days. These results were obtained with uniform regularity by all three observers, working independently, but employing the same general methods. The conclusions then drawn were of a tentative character, and it was deemed advisable to carry on further work. The studies here reported follow, in general, similar lines, using waters of different origin, under as widely diverse conditions as possible.

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[1] *Jour. Infect. Dis.*, 1904, 1, p. 641.

Special attention has also been given to the development of technical methods other than those previously used, so as to broaden, as far as possible, the basis upon which conclusions were to be made. Inasmuch as most of the technical methods used in the experiments here described are practically the same as those previously reported in the foregoing paper, it will not be necessary to repeat them in this connection. Only those modifications that further experience has demonstrated to be valuable, and the new methods that have been developed are here referred to.

These experiments have been made in the Wisconsin State Hygienic Laboratory at the University of Wisconsin. In part of the preliminary work much assistance was received from Mr. G. J. Marquette, then assistant in this laboratory. The waters used in these tests were from Lake Mendota, a spring-fed inland lake, of about 25 square miles extent, the waters of which may be regarded as fairly typical of those of a surface character. The sewage-infected waters were produced by adding to the lake water a given quantity of fresh liquid and solid human excreta.

METHOD OF EXPOSING THE TYPHOID BACILLUS.

In these experiments the exposure of the typhoid bacilli was made in the laboratory, rather than in the lake itself, the water, however, being piped for only a short distance. To place the infected sacs under conditions convenient for sampling and where they would not be subjected to the action of the weather, which was more or less troublesome in the Chicago series, the sacs containing the waters infected with the typhoid bacilli were placed in large tubulated glass receptacles, holding from two to three gallons, through which there was allowed to flow continuously a stream of water or sewage.

Reference to accompanying figure will indicate the arrangement of this device.

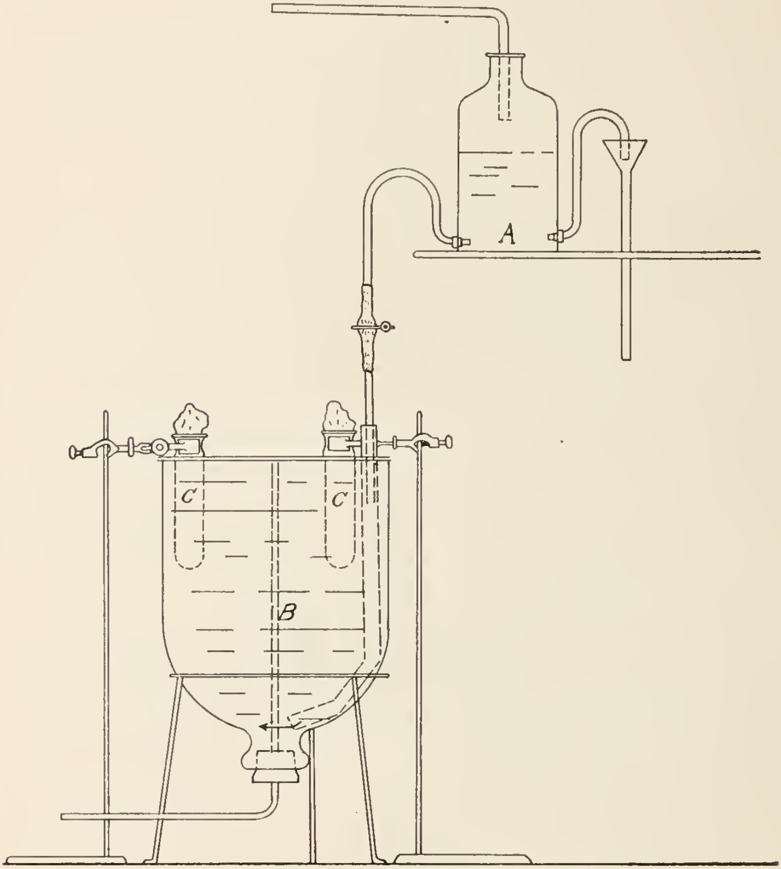

FIG. 1.

The sacs were immersed in the flowing stream so that the level of liquid in same was slightly below that of the water outside, thus maintaining a slight pressure toward the inside.

When exposed to the action of the lake water, the connection was made directly with the tap supplying this kind of water. To expose the sacs to the influence of sewage-infected waters, the device as shown in the cut was used. The sewage mixture was made up in a large reservoir placed in the attic, and from this was discharged through a pipe into a safety bottle, *A*, which regulated the flow into the reservoir below, *B*. The sacs, *C*, were held in position by clamping a rubber-faced clamp to a glass neck, which was sealed on to each kind of sac. The rate of flow was about six gallons per hour in the case of sewage, and a considerably higher rate for the lake water.

In the previous experiments, permeable sacs of celloidin and vegetable parchment were employed. In the work here recorded, another method has been devised, that of agar membranes. Some modifications of the previous methods have also been adopted.

1. *Celloidin sacs.*—The celloidin sacs employed have all been made by the extremely simple method of Frost,[1] in which the celloidin solution is poured on the inside of the test tube and the film, after it has been air-dried for the proper time, shrunk from the glass wall by means of water. By means of this method, sacs of practically any size can be made in a few minutes. These celloidin tubes were usually made to hold about 50 c.c. of water. They were held in position by inserting a glass neck of approximately the same bore as the sac, tying this on tightly with a soft-fibered thread, and coating the same with a layer of celloidin, allowing it to air dry. The sacs are filled with, as well as immersed in, water during the process of sterilization, which is done in an Arnold.

2. *Parchment sacs.*—In the former work sections of parchment tubing were used, such as is employed in dialysis work, but it is frequently difficult to secure tubing that is perfectly sound and free from minute holes. In this work, we have employed the parchment diffusion shells made by Schleicher and Schüll. These hold approximately 50 c.c. and are in the form of a tube closed at one end. Into the open, free end, a glass neck is fastened by means of sealing wax. The shells themselves are sterilized in streaming steam for an hour and a half, then allowed to dry under cover from the air. The glass necks can be sterilized chemically. The two parts can be quickly assembled in a sterile condition.

3. *Agar membrane sacs.*—The first introduction of agar for dialyzing purposes in bacteriological studies was made by Frost,[2] who used rectangular blocks about one-half inch square and an inch and a half long. These were made of plain agar and were inoculated by means of a stab in the center. The upper part of the stab was sealed off by dropping on melted agar, or smearing the upper surface of the block with a hot iron. Our first studies with agar were made with blocks of a similar character, but it was found that the

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[1] *Amer. Pub. Health Assoc. Rep.* 1903, 28, p. 36.

[2] *Jour. Infect. Dis.*, 1904, 1, p. 599

layer of agar was too thick to permit rapid and complete dialysis. In some cases the typhoid organism, inoculated as a stab culture, died in a short time, while in other cases it persisted for a long period (several weeks).

The attempt was then made to use an agar film instead of the thicker block. It is necessary to have some mechanical framework to support the thin, delicate film of agar, and for this purpose cellulose diffusion shells, such as are used in chemical manipulations, have been found very serviceable. These are of the same size as the parchment shells previously referred to (38 by 85 mm.). After they have been sterilized in steam and allowed to dry, sterile glass necks of the same bore are inserted into the shells and fastened by means of sealing wax. The shell is then ready to receive its agar coating. Care is taken in the preparation of the agar to remove from it as much organic matter as possible. This is done by soaking the thread agar in distilled water for some hours, changing the water several times. A 2 per cent solution is then made. Tests made as to the nutritive properties of this agar showed that it would not support bacterial growth. Occasionally molds will make a sparse growth on the medium when left exposed to the air for some time. In coating the shells with the agar film, the material should be used in as hot a condition as it can be handled, so as to impregnate thoroughly the pores of the cellulose filter. It is advisable to pour some of the hot agar on the inside of the sac, rotating the sac quite rapidly, as in a roll culture, so as to distribute the material uniformly. The porous cellulose wall absorbs the liquid rapidly. After a little experience, one learns the requisite quantity of medium to employ in order to give a uniform and sufficient coating, and not have an excess. It is advisable to have the coating made at a single immersion, as the film is more homogeneous than where several applications are made.

When the inside coating has been properly applied, the sac is then dipped into the liquid agar and rotated so as to coat the outside of the sac also with a uniform layer. If the inside of the sac has not been coated in such a way as to exclude the air entangled in the cellulose meshwork, air vesicles will develop in the outer agar coat after a time and the integrity of the sac will be destroyed. Care should be taken in this coating process to prevent infection of the sac, as it is of course impossible to sterilize the sacs after they are once made.

These agar films are not as durable as celloidin or parchment, but their integrity will be maintained unimpaired for about two weeks, or even longer, depending upon the nature of the liquid in which they are immersed. In water they retain their germ-tight properties longer than they do in sewage. In liquids very rich in bacteria, such as sewage, cytolytic enzymes are undoubtedly produced by certain types of organisms, thus softening the cellulose matrix and causing the sac to disintegrate.

While these agar membranes possess no point of superiority over the parchment or celloidin membranes formerly used, it is of importance to broaden the technical methods just as much as possible, and thereby determine if there are any essential variations in the results obtained which are due to the nature of the methods employed.

Permeability of different types of sacs used.—We have introduced into this study the use of a new type of permeable membrane, the agar film, and as no records have been reported on the question of relative permeability, so far as we know, it has been deemed advisable to incorporate here some of the results obtained in the study of these different types of sacs. The sacs used in this experiment are intended to hold in captivity the typhoid organism, and still at the same time subject this germ to the influence of diffusible substances that may be in the enveloping medium without.

In testing the permeability of these membranes, we have used simple, well-known substances that could be determined quantitatively, and have made no attempt to study the diffusibility of such materials as might possess an inhibitory effect on the imprisoned typhoid organism.

a) *Tests with chlorides.*—Experiments were first made with sodium chloride. Sacs were filled with distilled water and immersed in tap water, to which enough salt had been added to make the chlorine test 93 parts per million. Tests for chlorine were made on the contents of the immersed sacs at intervals of ½, 1, 6, 12, and 24 hours. The results of these determinations are expressed in Table 1.

From the results herein shown it appears that a condition of nearly perfect equilibrium was established in all three sacs within a comparatively short time. The diffusion of the chlorides was somewhat more rapid in the agar and celloidin sacs than in the parchment, but within 24 hours' time, where no artificial currents were

TABLE 1.

CHLORINE (PARTS PER MILLION) FOUND IN PERMEABLE SACS AFTER VARYING PERIOD OF IMMERSION IN SALT SOLUTION (93 PARTS PER 1,000,000).

	Agar	Parchment	Celloidin
½ hour	0	0	0
1 "	2.5	4	6
6 hours	63	50	76
12 "	80	67	84
24 "	84	76	86

used either within or without the sac to facilitate diffusion, from 81 to 92 per cent of the chlorine passed through the membranes of the sacs. In Fig. 2 are shown the data presented in the above table, expressed on a percentage basis of the total strength of the solution.

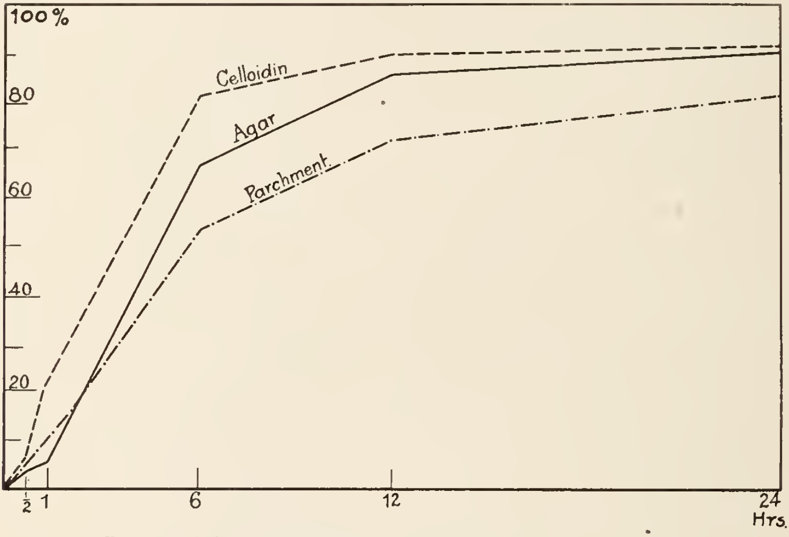

FIG. 2.—Relative permeability of different kinds of sacs to NaCl solutions

b) *Tests with sugars.*—In addition to the chlorides, tests were also made in the same way with sugar. Sacs filled with tap water were immersed in tap water containing 1.75 grms. of saccharose per 100 c.c. Quantitative determinations were made at ½, 1, 6, 12, and 24 hour intervals with the following results:

TABLE 2.

SUGAR (IN GRAMS PER 100 C.C.) FOUND IN PERMEABLE SACS AFTER VARYING PERIOD OF IMMERSION IN SOLUTION CONTAINING 1.75 GRAMS PER 100 C.C. WATER.

	Agar	Parchment	Celloidin
½ hour	0.03675	0.02625	0.20475
1 "	0.0630	0.0525	0.3850
6 hours	0.30475	0.22225	1.26525
12 "	0.6650	0.4270	1.4805
24 "	1.1375	1.0115	1.5925

These data, which are shown in graphical form in Fig. 3, seem to indicate that the celloidin type is much more permeable to sugar solutions than either agar or parchment. The rate of diffusion was not quite so rapid in this series as in the salt series, except in the case of the celloidin type.

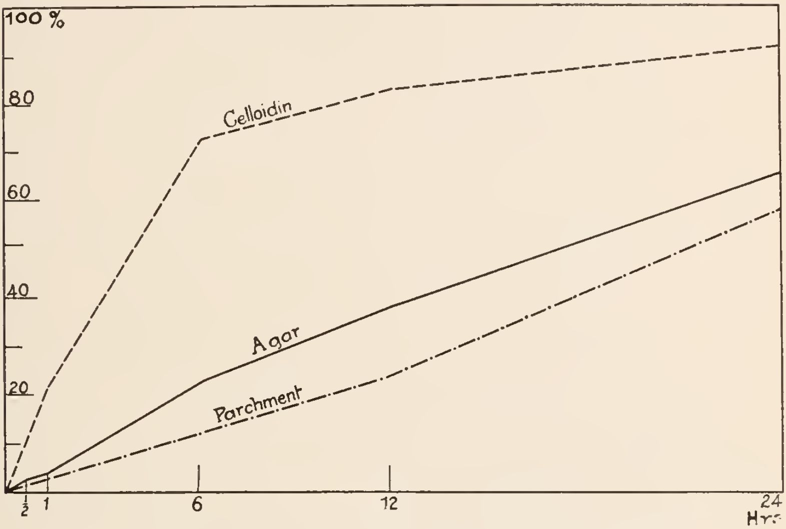

FIG 3.—Relative permeability of different kinds of sacs to sugar solutions.

c) Tests with peptone.—Further tests were made with peptone solutions in order to test the permeability of the sacs to diffusible proteids. Sacs were filled with tap water and immersed in the same type of water, containing 1 per cent peptone. In these tests, a qualitative determination only was made. The contents of the sacs were tested for peptone by means of the biuret reaction at intervals of five minutes, until a positive reaction was obtained. The celloidin sac gave a positive reaction in 25 minutes, while the agar and the parchment required 35 minutes. The intensity of the reaction increased markedly in all cases after an hour's exposure.

It would appear from these tests, where various chemical substances of a widely different nature were employed, that the permeability of these different membranes was pronounced. On the whole, the results indicate that celloidin is the best membrane to employ, but this type is well supplemented by the addition of agar and parchment. Further corroborative evidence on the matter of permeability may also be presented in results that were noted in the actual prosecution of the work.

Growth of bacteria in sacs immersed in nutrient solutions.—If sacs of this character are sufficiently permeable to permit food substances in solution to pass the limiting membrane, it ought to be possible to obtain growth of typhoid and water bacteria in sacs filled with water and immersed in liquids containing available organic matter.

To test this point more specifically, a special experiment was instituted. Six celloidin sacs were prepared and filled as follows: Two with sterilized tap water and the remaining four with raw tap water. The two sacs filled with sterilized water and two filled with raw tap water were inoculated with the same strength of typhoid suspension; the two remaining raw-water sacs were not infected with typhoid. These sacs were divided into two sets of three each. One set was immersed in running raw water, the other placed in sterile water containing 0.2 per cent peptone solution.

The results obtained in this experiment showed a most marked difference in the two sets of sacs, the peptone and the water series. In all three sacs of the peptone series a very marked growth was observed, both in the case of water bacteria originally present in the raw water, and the inoculated typhoid. The germ content of the control sac filled with raw water rose from a 32 colony count on plain agar on the first day, to 12,000,000 in the course of three days, and in 18 days had reached 40,000,000 bacteria per c.c. The sac filled with sterile water and infected with typhoid (16,000 per c.c.) underwent even a more pronounced growth than this. On the fourth day it contained 137,000,000 typhoid bacilli per c.c. From this high point the germ content gradually declined, but in 18 days there were still over 6,000,000 colonies per c.c., and the purity of the culture demonstrated the integrity of the sac. The course of changes followed by the sac containing the mixed flora (water bacteria + B. typhosus) underwent the same general change. On the 18th day 45,000,000 organisms per c.c. were demonstrable, and B. typhosus had been recovered in abundance on each intermediate day the test had been applied.

The course of changes noted in the water sacs was entirely different. The content of these sacs and the dosage was identical with the series immersed in the peptone solution. The only difference in this case was that the sacs were immersed in flowing tap water. The sac filled with raw water showed no growth on Drigalski-Conradi medium, and less than 50 bacteria per c.c. on plain agar.

Cultures were made for a period of six days, but no essential alteration in germ content was observed. The sac filled with sterile water and inoculated with B. typhosus (25,000 per c.c.) showed no increase. On the sixth day of the experiment it contained 17,000 organisms per c.c., apparently a pure culture (17 colonies picked all proved to be positive typhoids).

Accidentally the membrane was perforated on the seventh day, but for the period of observation reported no marked change had occurred in the germ content of the sac. The sac filled with raw water and infected with 30,000 B. typhosus fell to 3,000 on the third day. On the fifth day B. typhosus was recovered, but on the sixth day none could be found in culture plates containing about 100 colonies per c.c.

The results of this series are wholly consistent, and show that both water bacteria and B. typhosus are capable of multiplying extensively in raw waters, as well as sterile, where such waters are exposed in permeable sacs in liquids containing available food material in solution.

This experiment conclusively demonstrates the permeability of the sacs to such solutions, and would seem to show beyond all reasonable doubt that if bacteria die rapidly when imprisoned in such permeable cages, they do not succumb because of the inability of food substances in outside enveloping liquid to pass the limiting membranes of these sacs.

In addition to this carefully controlled experiment with peptone solutions, observations were made in the course of the experiments later detailed, which also throw light on this point. In series VIII a sac filled with raw water, but inoculated with typhoid, was immersed in a bath of flowing sewage. In Table 16 is shown the bacterial content of this sac on various days. A phenomenal development of the water bacteria occurred in this case, as in the peptone solution, showing that sewage also contains sufficient good material, which was able to permeate the celloidin membrane to give the water bacteria in raw water a favorable environment for rapid growth.

These results, taken in connection with the specific tests made as to permeability, would seem to indicate that the methods employed permitted diffusion to occur with sufficient rapidity so that the conditions approximated those that prevail in a flowing stream.

Germ-proof qualities of sacs.—In using sacs of a permeable character, it is of the utmost importance that they should be relatively germ tight. The celloidin sac has been tested for so long a period of time that there is no longer any question as to the tightness of sacs of this type, but concerning the use of parchment and agar, this question may well be raised. In our earlier experiments, the attempt was made to rely on parchment tubing, such as is used in dialysis work, but we have been much troubled to get satisfactory tubing of this character. The process of making the cellulose fiber into vegetable parchment seems to destroy the pliability of the material, so that it cracks more readily upon bending. In this way minute breaks or punctures are often to be noted. Since the adoption of the parchment diffusion shells, no trouble of this character has occurred.

The agar sacs are, of course, relatively fragile so far as the film is concerned, and the filter-paper matrix on which the agar is spread is not as permanent as parchment. When immersed in liquids rich in bacterial life, such as sewage, the cytolytic enzymes cause the disintegration of the cellulose fibers, this occurring more rapidly in the filter paper than with the parchment sac. In purer types of waters this rotting does not occur so readily. We have rarely had any trouble with sacs of this character, if the experiment did not exceed two weeks' time.

When the sacs are allowed to remain in dilute sewage, or even in water after a considerable lapse of time (10 days or more), a somewhat slimy growth is formed, as is also the case on the inner face of the glass receptacle. This is easily removed by brushing the sacs occasionally with a camel's hair brush.

Experiments were made with the special object of testing the integrity of these types of sacs. Sterile sacs were filled with sterilized water and immersed in either water or sewage. The result of such tests, even where continued for a week or more, showed no passage of bacteria through the sac membrane.[1]

[1] Johnson (*Eng. Rec.*, Sept. 23, 1905, also *Jour. New Eng. Water Works Assoc.*, 1905, 19, p. 508) has questioned the integrity of sacs of this character, but the experimental data given by this writer are too meager to permit of any weight being attached to his conclusions.

In some of the actual tests performed in these studies (see Series VII and VIII), it was possible to throw light on the matter of tightness of the sacs. Sacs filled with lake water and inoculated with typhoid were immersed in sewage. Drigalski-Conradi plates were made at daily intervals. If any leakage had occurred, it would have immediately manifested itself by the appearance of acid colonies on this medium, as, of course, the colon type was abundant in the dilute sewage outside of the sac. In most of the sacs no evidence of any leakage occurred. In one or two of them slight evidence of leakage was discovered in the course of 13 to 14 days. We are, however, of the opinion that any one of these three methods may be relied upon to maintain readily a germ-tight, and yet permeable, membrane, if any degree of care is used in selection and manipulation of the sacs.

CULTURES EMPLOYED.

In order that this work might be directly compared with that of the previous year, one of the same typhoid strains that was employed in the Chicago Drainage Canal work has been used throughout all of these experiments. This culture, strain "Y," was isolated October 1, 1903, under Professor Jordan's direction, from the urine of a typhoid patient. The case was typical in its symptoms, and the blood of the patient gave a positive agglutination test on the tenth day.

METHOD OF RECOVERY OF TYPHOID BACILLUS FROM SACS.

Where the typhoid organism is in direct contact with water or sewage forms, it is advisable to employ some of the special methods that have been devised for the differential cultivation of this organism. For this purpose we have employed throughout this work, the Drigalski-Conradi medium,[1] modified somewhat by the omission of nutrose. By filtering the mixture before adding the litmus solution, the flocculent precipitate is much reduced.

The use of a culture medium like the D.-C. medium is of great value in inhibiting bacterial growth which would otherwise obscure the typhoid organism. While the addition of the crystal violet does not completely inhibit water bacteria, it reduces materially the germ content, as is shown from the following data, where cultures were made on plain nutrient agar and on the D.-C. medium.

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[1] *Ztschr. f. Hyg.*, 1902, 39, p. 282.

TABLE 3.

RELATIVE GERM CONTENT OF WATER SACS ON AGAR AND ON DRIGALSKI-CONRADI MEDIUM.

Date	Amount Used	Medium Employed	Sac 1	Sac 2	Sac 3	Sac 4
1904 December 15........	1 c.c.	Plain agar	6,500	3,900	730	1,620
	1	D.-C. medium	750	410	150	350
December 16........	1	Plain agar	6,500	1,050	1,530	1,150
	1	D.-C. medium	560	300	137	175
December 17........	1	Plain agar	5,900	4,100	1,450	6,500
	1	D.-C. medium	1,150	4,300	490	150

RAPID IDENTIFICATION OF TYPHOID ORGANISM.

In determining whether any organism isolated from the plate cultures is true typhoid or not, it has generally been customary to test the presumptive organisms by first passing them through dextrose agar shake cultures, or stabs. From this, if no gas was produced, transfers were made into litmus milk and gelatin, and tests for indol were made. To differentiate it from B. alkaligenes, which develops no acid in litmus dextrose broth, cultures were made in this medium. If the organism in question stood all these culture tests satisfactorily for typhoid, it was then finally tested for agglutination with a highly potent typhoid-immune serum. Such a procedure as this involves a large number of transfers. In the course of these studies, a modification of the above method was made, which is as follows:

The Drigalski-Conradi plate cultures were carefully studied to pick out the presumptive typhoid colonies. This was not always easy to do, as there are many organisms occurring in sewage, or even in water, that are capable of development in this crystal violet medium, and which retain the blue color. Many of these can, of course, be easily rejected, as they are too luxuriant in their growth, being thick and opaque. But, not infrequently, types of colonies of a thin, semi-transparent blue cast have appeared, that more or less closely resembled the true typhoid. In picking out the presumptive typhoids, it is advisable to have on hand, for purposes of comparison, several culture plates made from a pure typhoid strain.

A more rapid method of identification was devised as follows: The presumptive typhoid-like colonies were fished and subcultured directly into litmus *dextrose* agar, by making a combination streak and stab culture. In this medium the typhoid, of course, formed acid, but no gas, and was thus easily differentiated from Petruschky's B. fecalis alkaligenes, which remained blue, not only in lactose but in glucose litmus media. Organisms of the colon type would naturally acidify dextrose agar, but these are excluded on the D.-C. culture plates. If, perchance, colon types should be transferred, their presence would be manifest by the copious gas production.

Following the litmus glucose test, all non-gas-producing acid forms were then subjected directly to the macroscopic agglutination test with typhoid immune sera. If the isolated cultures stood these two tests, they were considered positive typhoids. Upon the completion of these tests, a number of cultures from each series were selected at random and subcultured on all the usual media, as gelatin, milk, and glucose-free broth for indol, so as to check still further the culture characteristics of the supposed typhoid cultures.

Our experience with this short method of identification leads us to recommend its use over the longer method. We have found no organism in normal waters that is liable to be confused with the typhoid, where reliance is placed on these tests. In sewage, however, there is a blue type of organism that appears in 24 to 48 hours on Drigalski-Conradi plates, and which, therefore, might be transferred to the litmus dextrose cultures, on which it forms acid. This type has invariably failed to be agglutinated with typhoid-immune sera. Later, if one studies the original plate cultures, after a more prolonged period of incubation, he finds that these colonies are faintly acid. Evidently they are able to produce acid very slowly on lactose media.

OUTLINE OF EXPERIMENTS MADE.

In order to present a general summary of the work done in this series of studies, a synoptical table is presented below, in which the varying conditions, as to manner of exposure, dosage, temperature, etc., are shown.

TABLE 4.
SYNOPSIS OF DIFFERENT SERIES OF EXPERIMENTS MADE.

	No. of Series	No. Sacs Used	Kind of Sacs	Approximate Typhoid Dosage per c.c.	Nature of Liquid in Sac	Nature of Liquid in Outside Container	Range in Temperature (° C.)
LAKE WATER	I	2	Agar	110,000–200,000	Lake water	Lake water	10–14
		2	Celloidin	110,000–200,000	" "	" "	10–14
	II	1	Agar	25,000	" "	" "	9–12
		1	Celloidin	85,000	" "	" "	9–12
	III	1	Celloidin	150,000	" "	" "	15–18
		2	Parchment	120,000–150,000	" "	" "	15–18
	IV	1	Celloidin	2,275,000	" "	" "	21–23
		1	Glass	1,706,000	" "	....	21–23
SEWAGE	V	1	Parchment	1,500,000	Sewage	Sewage	21–29
		2	Celloidin	5,000,000	"	"	21–29
		1	Agar	10,000,000	"	"	21–29
	VI	1	Parchment	4,350,000	"	"	22–25
		1	Celloidin	5,800,000	"	"	22–25
SEWAGE IN WATER	VII	2	Celloidin	190,000–300,000	Lake water	Sewage	15–19
		2	Parchment	" "	" "	"	15–19
		2	Agar	" "	" "	"	15–19
	VIII	2	Celloidin	2,275,000	" "	"	22–25
		1	Parchment	1,706,000	" "	"	22–25
	IX	1	Celloidin	100,000	" "	"	22–25
		1	Parchment	200,000	" "	"	22–25
SEW'GE IN WATER	X	1	Parchment	4,350,000	Sewage	Lake water	21–23

PART I.

BACILLUS TYPHOSUS EXPOSED TO LAKE MENDOTA WATER.

Series I. Agar and celloidin sacs filled with lake water and immersed in lake water.— This series was started on December 5, 1904. Two agar sacs and two celloidin sacs were filled with raw lake water, and each sac inoculated with two large loopfuls of a 24 hour culture of the "Y" strain of the typhoid bacillus. These cultures were exposed continuously to the action of flowing lake water from the above date until December 22, making a period of 17 days in all.

The temperature of the water as it reached the exposed cultures was about 12° C. Quantitative plate cultures were made on the D.-C. medium throughout the whole experiment, in order to note the relative rate of decline of bacteria within the sacs. In the beginning three plates were made daily from each sac, using $\frac{1}{10}$, $\frac{1}{2}$, and 1 c.c. of the infected water, respectively. From this set of plates a very fair average could be determined, and the accuracy of the work thus checked by the quantitative findings with different dilutions. As the work progressed and the colony count per c.c. fell from the high initial number to lower limits, the quantity used in each seeding was materially increased. Under such conditions it is highly improbable that typhoid organisms were overlooked, where all typhoid-like colonies were removed and tested.

The quantitative findings of this series are presented in Table 5, from which it is apparent that the high initial content (which was, of course, largely composed of typhoid organisms at the beginning) underwent a rapid and continuous decline from the beginning to the seventh day. By this time the average number of organisms in the four different sacs had fallen from the initial seeding of 147,700 per c.c. to 2,170 per c.c. From this period to the end of the experiment, the number of organisms per c.c. remained very small, ranging at most from a thousand or so to a few hundred.

TABLE 5.

Number Bacteria per c.c. in Typhoid-Infected Sacs after Exposure to Flowing Lake Water.

Date of Test after Infection	Temperature	Sac 1 Agar	Sac 2 Agar	Sac 3 Celloidin	Sac 4 Celloidin
4 hours	12° C.	130,000	111,800	203,000	146,000
2d day	12	34,800	43,000	152,000	99,250
3d "	12	41,000	45,500	109,000	90,000
4th "	14	29,500	33,000	58,500	62,500
5th "	14	16,000	28,600	49,000	46,250
6th "	14	13.750	22,250	30.750	33,250
7th "	7	2.350	900	2,405	3,315
8th "	9	1,260	500	1,520	1,180
9th "	10	1,720	360	280	420
10th "	12	750	410	150	350
11th "	13	560	300	135	175
12th "	12	1,150	500	490	150

This comparative decline is also shown graphically in Fig. 4.

The marked diminution in numbers noted in the above table is unquestionably due to the rapid destruction of the typhoid bacilli inoculated into the raw water with which the sac was filled. The initial germ content of this water before inoculation with typhoid ranged from 500 to 1,000 bacteria per c.c., but unquestionably some increase in water bacteria would occur even in these permeable sacs. As the D.-C. medium was used throughout all these tests, these water bacteria would be largely prevented from development, so that the apparent diminution doubtless registers the decline in typhoid content.

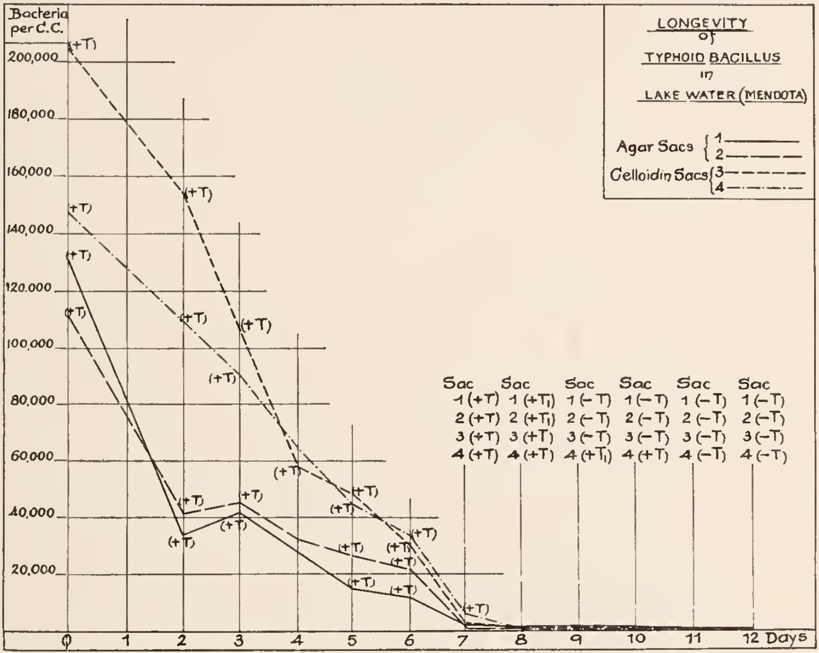

FIG. 4.

To check these quantitative results, extensive tests were also made to determine the longevity of the inoculated typhoid organisms by removing pure cultures from the Drigalski plates each day, and subculturing them.

The results of these qualitative tests are presented in Table 6, in which are given the number of organisms fished from the plates and also the number proven to be typhoid upon the basis of the agglutination test and subcultures. There was no difficulty in recognizing the typhoid organism in the plates during the earlier part of the series, as they were crowded with colonies of a similar character, and it was unnecessary to fish a large number. As the inoculated typhoid organism gradually died out, the colony appearance on the plates became more diverse, and the number of presumptive typhoid colonies was greatly diminished.

The results obtained in this series of tests are certainly very striking. A large proportion of the genuine typhoid colonies were found among those fished on the earlier days of the series. In the case of the agar sacs, as late as the seventh day, a majority of all fished colonies proved to be typhoid, while in the celloidin sac, this persistence was very marked, until a day later (eighth day). After

TABLE 6.

LONGEVITY OF B. TYPHOSUS IN AGAR AND CELLOIDIN SACS IN LAKE MENDOTA WATER

	SAC 1 (AGAR)		SAC 2 (AGAR)		SAC 3 (CELLOIDIN)		SAC 4 (CELLOIDIN)		TOTALS	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
4 hours.........	3	3	4	4	2	2	4	4	13	13
2d day.........	8	7	9	9	9	8	9	5	35	29
3d “	10	9	9	8	8	8	5	4	32	29
4th “	11	4	10	4	11	9	6	6	38	23
5th “	5	1	10	8	8	8	9	8	32	25
6th “	9	5	10	8	10	0	8	7	37	29
7th “	10	4	8	5	7	7	12	8	37	24
8th “	3	1	4	1	4	3	6	6	17	11
9th “	2	..	2	..	2	..	2	1	8	1
10th “	1	..	1	..	5	..	5	1	12	1
11th “	2	..	..	..	..	..	..	..	2	..
12th “	6	..	..	..	3	..	7	..	16	..
13th “	1	..	2	..	..	..	..	..	3	..
17th “	..	..	..	..	6	..	..	..	6	..
	71	34	69	47	75	54	73	50	288	186

these dates a very pronounced diminution in typhoid colonies appears. Scattering colonies were found in the agar sacs on the eighth day, but none later, and in one of the celloidin sacs a similar condition was observed on the 9th and 10th days, but none could be found subsequently. The appearance of the culture plates in the earlier and later periods of this series showed a marked difference in colony aspect. In the first week the plates were studded with apparently typically typhoid colonies, which upon subculture proved to be genuine typhoid by the different culture tests and the agglutination reaction. After this period (8 or 10 days), the aspect of the colonies appearing on the culture medium was of an entirely different character, and only rarely did any forms appear that could be suspected of typhoid relationships. In no case, however, did any of these organisms prove to be typhoid.

Series II. Agar and celloidin sacs filled with lake water and immersed in lake water.—This series, which was run prior to that just described above, is not as satisfactory, in that the data acquired are not nearly as complete as they should be, but it is presented as furnishing evidence of the action of lake waters under winter conditions. The series consists of two sacs, one agar and one celloidin, both of which were filled with raw lake water and inoculated with a typhoid dosage of 25,000 and 85,000 typhoid bacilli per c.c., respectively. The sacs were then immersed, as before, in flowing lake water, having a range in temperature from 9° to 12° C. The data as to the presence of the inoculated typhoid bacilli are presented in Table 7.

TABLE 7.

LONGEVITY OF B. TYPHOSUS IN AGAR AND CELLOIDIN SACS IN LAKE MENDOTA WATER.

	Temp. ° C.	Sac 5 (Agar)		Sac 6 (Celloidin)		Totals	
		No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
3 days....................	14	7	4	10	2	17	6
6 "	12	6	0	2	0	8	0
8 "	12	11	4	9	1	20	5
12 "	14	4	0	5	0	9	0
		28	8	26	3	54	11

While the number of colonies removed in this case was not large, yet the same general result was obtained as before. It was readily observable on the plates made the third day after seeding that the number of typhoid colonies had undergone a marked reduction. While a daily study of this series was not made, yet the results, as far as they go, confirm in general the conclusion of the preceding series. A marked reduction in total colony count was observable after the third day. The last time typhoid was found was on the eighth day.

TABLE 8.

LONGEVITY OF B. TYPHOSUS IN PARCHMENT AND CELLOIDIN SACS IN LAKE MENDOTA WATER.

	Sac No. 7 (Parch.)		Sac No. 8 (Parch.)		Sac 9 (Cell.)		Totals—All Sacs	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
1 hour.........	4	4	4	3	4	3	12	10
1 day..........	6	0	6	2	4	4	16	6
2 days.........	5	1	8	1	4	2	17	4
3 "	8	2	8	0	5	5	21	7
4 "	5	2	8	1	7	0	20	3
6 "	6	2	10	4	7	1	23	7
8 "	10	1	6	0	9	0	25	1
10 "	4	0	3	0	3	0	10	0
11 "	4	0	3	0	3	0	10	0
13 "	4	0	3	0	1	0	8	0
15 "	1	0	..	..	1	0	2	0
17 "	1	0	2	0	..	..	3	0
	58	12	61	11	48	15	167	38

Series III. Parchment and celloidin sacs filled with lake water and immersed in lake water.—Another series in lake water was begun on May 2, 1905, in which the same general arrangement as before was followed. One celloidin and two parchment sacs were immersed in lake water, after being filled with lake water, which at this time contained 140 bacteria per c.c. The seeding of typhoid in this case ranged from 120,000 to 150,000 bacteria per c.c. Determinations made of the germ content of the lake water during the progress of the experiment, which lasted from May 2 to May 17, showed at all times less than 400 bacteria per c.c. The temperature under these summer conditions ranged from 15° to 18° C., and was materially higher than in the two preceding series. The results of this test are shown in Table 8.

Here again there is practical unanimity as to the results obtained in the parchment and celloidin sacs. Typhoid colonies were detected in all three sacs on the sixth day and in one sac as late as the eighth day, although only one colony was found. Cultures were continued for a period of 17 days, but no typhoid colonies were recovered after the period mentioned. In this series the typhoid type could be differentiated on the culture plates with a greater degree of accuracy than in the preceding Series I. An attempt was made to estimate, only approximately, of course, the number of typhoid colonies that developed on the various plates. These data cannot be relied upon implicitly, because one cannot be absolutely sure as to whether a colony is typhoid or not, where reliance is placed on the culture-plate appearance. But the plates were held in the ice box until after the first picking had been run through the necessary differential tests, so that the differentiation was more accurate than it otherwise would have been. The following records give the approximate number of typhoid colonies on the various plates for the different days of exposure.

Date	Days of Exposure	Character of Culture Plates
May 2	0	Typhoid type greatly predominating
May 3	1	Total colonies much diminished, about 8,000 typhoids per c.c.
May 4	2	About 700 typhoids on 1 plate; less on other 2 cultures
May 5	3	1 plate ($\frac{1}{10}$c.c.) had clump of about 50 typhoids, besides other scattering colonies. Both other plates contained a few
May 6	4	Several score typhoids on plate 1. 20–30 each on plates 2 and 3
May 8	6	40 typhoids on parchment sac from which 4 fished colonies were proven to be genuine typhoid. Only sporadic colonies on plates of other sacs
May 10	8	Character of plates completely changed. No typhoid observed except the single colony isolated from parchment sac
May 12	10	$\frac{1}{4}$ and $\frac{1}{2}$ c.c. water now used in culture plates. Germ content 300–500 colonies per plate, but all negative typhoid
May 15	13	1 c.c. cultures. Germ content trivial on D.-C. plates
May 19	17	1 c.c. cultures. D.-C. plates nearly sterile. Lactose agar about 150-200 colonies per c.c.

Thus, from an ocular inspection of the culture plates, as well as from the completed study of the isolated cultures, death is to be noted, in the course of a few days, of the hundreds of thousands of typhoid bacilli introduced into the lake water at the beginning. The great majority of these organisms disappeared in the course of a few days (three or four), and after the lapse of six days, they could only be found in sporadic cases.

Series IV. Celloidin sac and glass tube filled with lake water, and immersed in lake water.—This series, run from August 15–24, 1905, included a celloidin sac and a glass tube of similar size. The dosage of the two containers was quite heavy, 2,275,000 and 1,706,000 typhoid bacilli per c.c. respectively. The temperature ranged from 21° to 23° C. during the work.

TABLE 9.

Longevity of B. typhosus in Celloidin Sac and Glass Container in Lake Mendota Water.

	Sac 10 (Celloidin)		Sac 11 (Glass Container)		Totals	
	No. Col. Fished	No. Proven Typhoid	No. Col. Fished	No. Proven Typhoid	No Col. Fished	No. Proven Typhoid
1 hour	17	11	10	10	27	21
2 days	15	5	4	4	19	9
4 "	29	19	7	3	36	22
6 "	8	0	17	0	25	0
7 "	12	0	18	6	30	6
8 "	25	2	14	1	39	3
10 "	7	2	30	6	37	8
13 "	6	0	18	1	24	1
14 "	4	0	22	0	26	0
	123	39	140	31	263	70

In this series in the permeable sac, the inoculated typhoid was found as late as the 10th day, while in the glass container, immersed in the reservoir so as to maintain exactly the same temperature, it persisted until the 13th day. This result is in accord with data previously collected, where experiments have been carried on in glass receptacles, in which case it is generally found that the longevity of the typhoid organism is materially prolonged. For this reason, the earlier work on this question of longevity cannot be regarded as conforming to conditions that obtain in nature.

SYNOPSIS OF EXPERIMENTS WITH TYPHOID BACILLI EXPOSED TO NORMAL LAKE WATER.

In the foregoing series (I–IV) there is, on the whole, a striking agreement as to the length of time that the typhoid organism could be detected. In two respects considerable variation was to be observed in these series, viz., temperature of exposure and dosage, but the longevity of the introduced organism ranged through a comparatively narrow period of time (8–10 days). Two of these series were carried out in the winter, one in the spring, and another in the summer, when the average temperature of the water ranged from 21° to 23° C. In Series III, which was made in May, the organism persisted for the minimum period of time, and it was thought possible that this might be ascribed to the higher temperature; but in Series IV, where the temperature was still higher, B. typhosus persisted for the usual period (8–10 days). In this case, though, the typhoid dosage was high, as the sacs were inoculated with approximately 2,000,000 organisms per c.c., a number much larger than usual.

In Table 10 is presented a summary of the results obtained in all these series, and, for purposes of comparison, the data collected last year by Professor Zeit in the work on Lake Michigan are also incorporated.

TABLE 10.

SUMMARY OF EXPERIMENTS ON LONGEVITY OF B. TYPHOSUS EXPOSED TO SURFACE WATERS (LAKE MENDOTA AND LAKE MICHIGAN).

LAKE MENDOTA.

SERIES	KIND OF SAC	SAC NO.	DAYS																		TOTAL NO. COL. FISHED	NO. PROVEN TYPHOID COL.
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
I	Agar..........	1	+		+	+	+	+	+	+	+	o	o	o	o	o				o		
	"	2	+		+	+	+	+	+	+	+	o	o			o				o		
	Celloidin......	3	+		+	+	+															
	"	4	+		+	+	+	+	+	+	+	+1	+1		o					o	288	186
II	Agar..........	5				+			+		+				o							
	Celloidin......	6				+			o		+1				o						54	11
III	Parchment....	7	+	o	+	+	+		+		+1		o	o		o		o		o		
	"	8	+	+	+	o	+		+		o		o	o		o				o		
	Celloidin......	9	+	+	+	+	o		+1		o		o	o		o		o			167	38
VI	Celloidin......	10	+		+		+		o	o	+		+			o	o					
	Glass.........	11	+		+		+		o	+	+		+			+	o				213	63

LAKE MICHIGAN (ZEIT).

SERIES	KIND OF SAC	SAC NO.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TOTAL NO. COL. FISHED	NO. PROVEN TYPHOID COL.
	Parchment	..	+	+	+		+		+	+	o		o	o								
	"	..	+	+	+	+		+			o	o										
	"	..	+	+	+	+		+		+	o		o		o							
	"	..	+	+	+	+	+	+	o	o	o	o				o						
	"	..	+	+	+		+	+	+	o	o	o										
	Celloidin.......	..	+	+	+	+	+	+	+	+	o	o	o	o	o	o						

+ means that typhoid was found more or less abundantly; o indicates complete disappearance of typhoid; +1, where figure "1" follows the plus sign, signifies that the positive typhoid findings were reduced to a single colony.

Comparing these two types of surface waters, one from an inland lake of moderate dimensions, the other from a very much larger water reservoir, it appears that the results of these two sets of experiments are not greatly different. In Professor Zeit's work, the average period of longevity was about seven days, while in our studies it has ranged from 8 to 10 days. Still, by far the most of the typhoid organisms disappear before the end of a week. It is, however, necessary to set the limit at complete disappearance, although it has been generally noticed that there are often a few seemingly more resistant individual germs that persist for an appreciably longer time than the average.

PART II.

BACILLUS TYPHOSUS EXPOSED TO DIRECT INFLUENCE OF FRESH SEWAGE.

Having determined the relation of the typhoid organism to such natural surface waters as those of Lake Mendota, in which the normal bacterial content is relatively low, we next directed our attention to the question of the longevity of this organism when exposed to the influence of liquids rich in germ life and their products of growth. The previous work[1] on the waters of the Chicago Drainage Canal had indicated that the typhoid bacillus was unable to survive in highly polluted waters for as long a period of time as when exposed to a purer type of surface waters. This conclusion was made in a tentative way, but the importance of it in sanitary work is such that further study is desirable.

The purpose of the following series was to repeat this work on sewage in order to test the validity of the tentative conclusion previously drawn. For this purpose sacs were filled with fresh sewage, inoculated heavily with the same strain of the typhoid bacillus previously used, then immersed in a reservoir through which a stream of fresh sewage was allowed to flow.

Series V. Parchment, celloidin, and agar sacs filled with sewage and immersed in flowing fresh sewage.—Under the conditions of this series, the typhoid bacillus was exposed to the direct influence of the sewage organisms themselves, as well as their by-products of growth. In carrying out these experiments, it was necessary to use a much wider range of dilutions in making the cultures, in order to give the introduced bacillus most favorable opportunities for development. The typhoid dosage used was naturally much larger than that employed in the preceding cases. As was customary with the plate cultures made in the previous series, all plates were saved after they had been subjected to the usual examination, and all presumptive typhoid colonies marked and subcultured. The plates were then allowed to develop further, and after the discontinuance of sampling, the entire series, as a whole, was subjected to a comparative study, and the second crop of typhoid-like colonies removed. By subjecting the plate cultures to this comparative study, it is believed that it was possible to locate all typhoid organisms that developed on the plates.

In this series, three sacs (one each of celloidin, agar, and parchment), were filled with sewage and then immersed in flowing sewage. These sacs received respectively 1,500,000, 5,000,000, and 10,000,000 bacteria from a 24 hour culture of the "Y" strain.

Another celloidin sac, filled with sewage of the same composition, but not inoculated with typhoid, served as a control to study the course of the bacterial changes in the sewage itself.

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[1] *Jour. Infec. Dis.*, 1904, 1, p. 641.

The sewage solution was made by mixing fresh human excreta (liquid as well as solid) with lake water and holding the mixture in a reservoir containing about 35 gallons. This reservoir was filled from time to time to maintain a continuous flow. As judged by appearance and odor, the sewage was fairly strong. Chlorine determinations were made at intervals, but naturally there was considerable fluctuation, depending upon the introduction of the urine. At the beginning of the experiment the sewage contained 244 parts of chlorine per million, while at the end there were 340 parts. The chlorine content was, however, lower than this during the progress of the experiment.

The following observations were made on the germ content of the sewage in the outside reservoir and within the control non-typhoid-infected sac.

TABLE 11.

BACTERIAL CONTENT PER C.C. OF SEWAGE.

	DAYS OF EXPOSURE	OUTSIDE FLOWING STREAM		INSIDE OF CONTROL SAC
		Lactose Agar	Drigalski-Conradi Medium	Lactose Agar
July 13	0	7,875,000	3,500,000	10,500,000
July 15	2		250,000	6,350,000
July 17	4		5,185,000	5,150,000
July 26	13		7,100,000	

In all cases an abundance of acid colonies on the D.-C. medium indicated the presence of sewage types. The temperature of the flowing liquid ranged from 21° to 29° C., with an average for the whole period of 24.8° C.

Results obtained in series V.—The examinations made on this series were begun on July 13 and continued on most of the sacs till July 27, covering a period of 14 days. At this time the character of the culture plates indicated that the typhoid type had entirely disappeared, and from previous experience it was deemed inadvisable to continue sampling longer. The results of this series are briefly summarized in Table 12, in which are given, for the respective days, (1) the total number of colonies picked, (2) the number which were regarded as presumptive typhoids on the basis of the litmus glucose agar test, and (3) the number of proven or verified typhoid colonies as determined by the agglutination and the differential culture tests.

The results obtained in all three sacs, including parchment, agar, and celloidin types, are in striking agreement with each other. In the three different kinds of permeable sacs employed, the results were identical. The last typhoid organism was found in each sac on the fifth day of exposure, but it is noteworthy that a marked decline

TABLE 12.

LONGEVITY OF B. TYPHOSUS EXPOSED IN PERMEABLE SACS FILLED WITH SEWAGE AND IMMERSED IN FLOWING SEWAGE.

DAYS	SAC 12 (AGAR)			SAC 13 (PARCHMENT)			SAC 14 (CELL.)			TOTALS DIFF. DAYS		
	No. Col. Fished	No. Pres. Typh. Found	No. Prov'n Typhoid	No. Col. Fished	No. Pres. Typh. Found	No. Prov'n Typhoid	No. Col. Fished	No. Pres. Typh.	No. Prov'n Typhoid	No. Col. Fished	No. Pres. Typh.	No Prov'n Typhoid
0	19	10	6	..	..	..	..	..	..	19	10	6
1	13	3	3	36	29	28	19	7	6	68	39	37
2	16	9	9	10	6	6	11	3	3	37	18	18
3	..	..	..	7	2	2	10	4	1	17	6	3
4	27	9	1	20	5	1	26	6	1	73	20	3
5	24	1	1	36	1	1	27	2	2	87	4	4
6	17	2	0	8	1	0	17	0	0	42	3	0
7	7	2	0	17	0	0	8	0	0	32	2	0
8	10	0	0	6	0	0	10	0	0	26	0	0
12	5	0	0	2	0	0	1	0	0	8	0	0
	138	36	20	142	44	38	129	22	13	409	102	71

set in considerably earlier. After 24 hours' exposure, of the 39 presumptive colonies removed from the D.-C. plates, 37 proved to be genuine typhoid. This percentage was maintained in equal ratio on the second day, but after this date fell rapidly, so that from the third to the fifth day of exposure, there could be found on cultures from each sac only one or two colonies that proved to be B. typhosus. After this date, 108 more colonies were taken off, but nearly all were eliminated by the litmus glucose test, and all proved negative typhoid on the application of the agglutination test.

TABLE 13.

LONGEVITY OF B. TYPHOSUS EXPOSED TO THE DIRECT ACTION OF SEWAGE BACTERIA.

DAYS	SAC 15 (PARCHMENT)			SAC 16 (CELLOIDIN)			TOTALS		
	No. Col. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.
0	16	16	16	..	..	..	16	16	16
1	3	3	3	50	7	7	53	10	10
2	38	15	15	26	3	3	64	18	18
3	22	10	10	20	2	2	42	12	12
4	15	0	0	8	0	0	23	0	0
5	7	0	0	13	0	3	20	0	3
6	7	0	0	6	0	0	13	0	0
8	3	0	0	2	0	0	5	0	0
10	3	0	0	5	0	0	8	0	0
14	3	0	0	4	0	0	7	0	0
16	1	0	0	7	0	0	8	0	0
	118	44	44	141	12	15	259	56	59

Series VI. Parchment and celloidin sacs filled with sewage and immersed in sewage.—The same method of arrangement, as detailed in preceding series, was followed in this series, which was begun on August 15, 1905, and continued until

August 29. A parchment and a celloidin sac, heavily seeded with typhoid organisms (4,350,000 and 5,800,000 bacteria, respectively), were filled with fresh sewage and immersed as before in flowing sewage. Bacteriological examinations made with D.-C. media on the sewage showed usually a germ content ranging from 1,250,000–6,600,000. The chlorine content varied from 150 to 200 parts per million. The range in temperature was from 22° to 25° C. The results obtained are shown in Table 13.

In this series the disappearance of the typhoid organism was even more rapid than in the foregoing test. Two hundred and fifty-nine cultures were removed, and of these 59 proved to be typhoid. In the parchment sac none were found after the third day, while in the celloidin sac the presumptive typhoids were sparse after two or three days, and had entirely disappeared after five days.

These results are in harmony with those obtained the year before on the Chicago Drainage Canal, in that in both sets of experiments, the longevity of the typhoid organism was much shorter when exposed in sewage than in lake water. In the earlier series of studies, the introduced organism was not found after the third day, while practically the same result was obtained in both of the series here recorded, although scattering colonies were found on the culture plates as late as the fifth day.

Taking into consideration, the results obtained in both the Chicago series and those here described it would seem that the data obtained warrant the conviction that the typhoid organism is unable to retain its vitality as long when immersed in sewage as it does when in contact with lake water. This fact being determined, the next question of interest is to find the cause of this phenomenon. Is this diminished longevity due (1) to the direct action of the sewage bacteria themselves, or (2) to the by-products which this type of life produces? By means of the technical methods here used, it was thought that this problem might be solved by exposing the typhoid organisms in sacs filled with lake water to the influence of flowing sewage. If the period of longevity of the inoculated organism was fully as great when the lake water sacs were immersed in sewage as when subjected to the current of flowing lake water, then it would seem that the influence of the soluble growth products of sewage bacteria would be of no effect. On the other hand, if they died as quickly as they did in sewage, or nearly as soon, then it would appear that the soluble substances passing from the sewage outside through the permeable membranes exerted a harmful action.

To test this hypothesis, several series were instituted in which the inoculated organism was exposed in the way just mentioned.

PART III.

BACILLUS TYPHOSUS EXPOSED TO THE INFLUENCE OF DIFFUSIBLE SEWAGE BY-PRODUCTS AND TO WATER BACTERIA.

Three different sets of experiments were made on this point, in which sacs were filled with raw lake water, then inoculated with B. typhosus and the whole immersed in flowing sewage. Series VII, consisting of two sacs each of celloidin, parchment, and agar, was inoculated with 190,000–300,000 organisms per c.c. and run from May 2 to May 19 of this year.

Two other series (VIII and IX), each consisting of a celloidin and a parchment sac, were inoculated, one with a heavy seeding, the other with a light seeding, of B. typhosus, and immersed in flowing sewage.

Series VII. Celloidin, parchment, and agar sacs, filled with lake water and immersed in flowing, fresh sewage.—Six sacs were employed in this series, two each of the three types. The sewage on the outside of the sacs was fairly strong, as judged by sight and smell. It was quite turbid, and of a dark brown color, due to accumulation of organic matter, which collected on the walls of the containing reservoir, especially at and near the surface. A cover-glass preparation showed a matrix of colorless filaments, brownish cells, and amorphous matter. From time to time the germ content and the chlorine content of this flowing liquid was ascertained to gauge its relative condition. The bacterial counts were made after 24 hours' incubation. A longer period of incubation would doubtless have increased materially the figures given.

TABLE 14.

CHARACTER OF SEWAGE AS TO CHLORINE AND BACTERIAL CONTENT.

	Chlorine Pts. per 1,000,000	Bacteria per c.c. Lactose Agar	Litmus Lactose Agar
May 2	...	128,250	Numerous acid and gas colonies
May 4	175	......	
May 5	...	80,000	Gas formers and acid colonies abundant
May 6	125	......	
May 8	22	61,500	Sewage forms abundant
May 10	...	160,000	Acid and gas colonies numerous
May 11	15	......	
May 12	24	......	
May 13	27	.....	
May 15	24	......	
May 17	20	......	
May 18	70	.....	

The variation in chlorine content was doubtless occasioned by the somewhat irregular addition of the urine to the sewage. The temperatures ranged in this series from 15° to 19° C., with an average temperature of 16.7° C. All three kinds of sacs were used in this series, two sacs of each being employed. At the outset an attempt was made to determine the quantitative condition of the respective sacs in a manner similar to that indicated in Series I, but this was discontinued during the progress of the experiment when it became evident that a large proportion of the organisms found on the culture plates were not typhoid, but were water saprophytes. The germ content of the different sacs at the beginning ranged from 190,000 to 330,000 per c.c., which was, of course, mainly B. typhosus, as the water used in the sacs was lake water, containing not more than 150–300 bacteria per c.c. On the second and third days of exposure the total germ content in the different sacs had fallen 50–90 per cent, but after this date a marked increase occurred, reaching in the course of a few days several hundred thousand bacteria per c.c. It would appear from these data that the water bacteria originally introduced, with the lake water, into the sac, had multiplied extensively. This is a matter of import, as this multiplication might exert some effect upon the longevity of the pathogenic organism with which they were exposed.

A more important fact, however, is that this demonstrates the permeability of the sacs to substances from without, for this enormous development far transcends any growth that would have occurred without influx of organic matter from the outside. That none of this growth was due to the admission of the sewagic forms from the outside is conclusively shown by the entire absence of any gas forming acid colonies on all the plates for about two weeks. The colonies growing on the Drigalski-Conradi medium were uniformly of some shade of blue or grayish color. Not until the 13th day did any acid colony appear. On this day a single acid colony developed in cultures made from one of the parchment, and also from one of the agar, sacs. This condition was also observed the next day in the same parchment sac, and in the other agar sac. These results, therefore, speak strongly for the integrity of the sacs for a sufficiently long period, as they were surrounded continually with water containing hundreds, and often thousands, of acid and gas generating forms per c.c.

The records of the plates as to their colony appearance is herewith presented:

Date	Days of Exposure	Character of Culture Plates
May 2	0	All sacs cultured immediately after installation and found to contain very nearly a pure typhoid culture
May 3	1	Major portion of colonies typhoid on parchment (Sac 18); agar sac (Sac 22) very nearly a pure culture of typhoid
May 4	2	Presumptive typhoids apparently few on agar (Sac 21). Suspected colonies picked failed to grow
May 5	3	About one-half on celloidin (Sac 19) appear to be typhoid. Typhoid abundant on agar (Sacs 21 and 22)
May 7	5	Typhoid abundant on celloidin (Sac 19)
May 8	6	Typhoid quite numerous on celloidin (Sac 20)
May 9	7	Culture badly mixed. Suspected typhoids very sparingly present
May 10	8	Colonies on plates from agar sac appear negative typhoid
May 11	9	Several colonies on celloidin (Sac 19) resemble typhoid, but only one proves positive
May 13	11	All colonies evidently negative typhoid. Total colony count falling rapidly

The results obtained from the testing of the cultures fished from the culture plates are expressed in Table 15.

TABLE 15.

LONGEVITY OF B. TYPHOSUS IN PERMEABLE SACS FILLED WITH LAKE WATER AND EXPOSED TO INFLUENCE OF FRESH SEWAGE.

	SAC 17 PARCH.		SAC 18 PARCH.		SAC 19 CELL.		SAC 20 CELL.		SAC 21 AGAR		SAC 22 AGAR		TOTALS	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Proven Typh.
1 hour	6	6	4	3	3	2	3	3	5	5	4	4	25	23
1 day	3	1	7	7	3	3	3	3	5	3	4	4	25	21
2 days	4	1	..	..	5	2	7	2	4	0	5	0	25	5
3 "	4	0	8	4	5	4	2	2	4	1	3	2,	26	13
4 "	6	2	..	..	4	2	7	4	1	1	6	1	24	10
5 "	6	1	7	0	10	7	..	..	15	2	..	..	38	10
6 "	..	..	..	..	..	..	7	5	..	..	6	2	13	7
7 "	10	2	3	0	3	0	..	..	5	0	..	..	21	2
8 "	..	..	6	0	..	..	7	2	..	..	5	1	18	3
9 "	7	0	5	0	9	1	..	..	4	0	6	..	25	1
10 "	..	..	1	0	..	..	4	0	..	0	5	0	10	0
11 "	5	0	..	..	5	0	4	0	4	0	6	1	24	1
13 "	4	0	..	..	5	0	5	0	3	..	3	0	20	0
15 "	2	0	1	0	1	0	1	0	1	..	1	0	7	0
17 "	..	..	..	..	4	0	5	0	..	..	..	..	9	0
	57	13	42	14	57	21	55	21	51	12	48	15	310	96

In the parchment series, the character of the colonies in the culture plates had changed by the seventh day so that typhoid-like organisms were very sparingly present. The results in all of the sacs, as a whole were, however, more divergent than in any of the preceding series. An average of the whole six sacs used was something over seven days, not greatly different from those made in flowing lake water. Even though the divergence in results was considerable, the sacs in this series showed a greater degree of longevity than in those in which the typhoid organisms were immersed in sewage. This would seem to indicate that the sewage in the outer reservoir exerted little or no effect on the vitality of the typhoid within. With such divergent results it is, however, impossible to draw any conclusion, and the further data on this point, presented in Series VIII and IX, are necessary as a basis for deductions.

Series VIII. Celloidin and parchment sacs filled with lake water and immersed in flowing sewage.—This series consisted of three sacs: one celloidin and one parchment, filled with raw lake water and inoculated with the usual typhoid strain; also, a third celloidin sac filled with lake water, but uninfected with the pathogenic organism. These three sacs were immersed in a bath of flowing sewage. The exposure was continued for a period of 14 days. The temperature ranged from 21° to 29° C., with an average of about 25° C. The sewage in the outside receptacle was fresh and quite strong. Chlorine determinations showed a range from 241 to 340 parts per million. The bacterial content of this outside sewage was usually from 3,500,000 to 7,000,000 organisms per c.c.

A bacterial determination on Drigalski-Conradi medium was made of the uninfected celloidin sac, and it is interesting to note the enormous development that occurred in the lake water submerged in the sewage, as shown in the following data.

TABLE 16.

BACTERIAL CONTENT PER C.C. OF LAKE WATER IN CELLOIDIN SAC IMMERSED IN FLOWING SEWAGE.

	Period of Immersion (Days)	Bacteria per c.c.	Acid Colonies
June 13	0	30	0
June 15	2	10,500	0
June 18	5	10,950,000	0
June 25	12	26,600,000	0

The above data are of importance, as showing the course of the changes that occur in the permeable sacs when immersed in a medium containing a large amount of organic matter. This sac filled with water showed a degree of growth that is almost unparalleled, multiplying in the course of 12 days nearly a million-fold. The fact that no acid colonies developed on these plates made from the water is proof of the integrity of the sacs. This bacterial growth is far in excess of that which occurs in the permeable sacs when immersed in flowing lake water, and would seem to be explained on the assumption that nutrient substances of a diffusible character are capable of passing through the membrane, from the sewage outside.

The record of the longevity of the typhoid type is shown below.

TABLE 17.

LONGEVITY OF B. TYPHOSUS IN CELLOIDIN AND PARCHMENT SACS FILLED WITH LAKE WATER AND IMMERSED IN FLOWING SEWAGE.

	SAC 23 (PARCHMENT)		SAC 24 (CELLOIDIN)		
	No. Colonies Fished	No. Proven Typhoid	No. Colonies Fished	No. Presumptive Typhoid	No. Proven Typhoid
0 days	..	..	13	12	10
1 day	9	0			
2 days	6	0	9	0	0
4 “	13	5	10	8	8
6 “	18	9			
10 “	21	4	Leak discovered, further sampling discontinued		
11 “	22	0			
12 “	17	0			
13 “	15	0			
14 “	16	0			

In this series an accident happened to the celloidin sac. In some way a leak developed, which fact could of course be quickly detected by the appearance of red colonies on the D.-C. plates, whereas the lake water, to begin with, was free from all acid-producing forms. From Sac 23 it happened that no cultures were made between the 6th and the 10th days, so that the history at this point is not as complete as it should have been, but it is significant that the typhoid organisms were readily recovered on the 10th day. A large number of cultures were taken after this date (70 on four successive days), but in no case was any organism found that even simulated the typhoid type of colony or proved positive upon the application of the differential tests.

When these results are compared with those contained in Series VII, it appears in both series that the inoculated typhoid persisted for a considerably longer period than in case of direct contact with sewage itself.

A rapid change, as usual, took place in the general character of the culture plates. On July 14, the second day after the lake water was infected, the major portion of the plate cultures showed typical typhoid colonies. In the course of a few days the total germ content per c.c. of this sac increased rapidly, due to the growth of the water organisms. On the 17th, five days after starting the experiment, about one-third (16,000 organisms) of the total colony count was still B. typhosus. By the 19th this number had fallen to about 8,000 per c.c. On the 23d both D.-C. plates contained a few presumptive typhoids, but the later plates showed totally aberrant forms.

Series IX. Celloidin and parchment sacs filled with lake water and immersed in flowing sewage.—This series was started on August 15 and continued till the 29th. Two sacs, one of celloidin and the other of parchment, were inoculated lightly with

100,000 and 200,000 typhoid bacilli, respectively. The findings in this test are expressed in Table 18.

TABLE 18.

LONGEVITY OF B. TYPHOSUS IN SACS FILLED WITH LAKE WATER AND EXPOSED TO THE ACTION OF FLOWING SEWAGE.

Days	Sac 25 (Celloidin)			Sac 26 (Parchment)			Totals		
	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.
0.............	12	12	12	14	14	13	26	26	25
2.............	12	2	2	12	1	1	24	3	3
4.............	17	0	0	21	1	1	38	1	1
6.............	26	11	11	30	11	11	56	22	22
7.............	10	6	6	59	14	14	69	20	20
8.............	21	0	0	31	1	1	52	1	1
10.............	3	0	0	5	0	0	8	0	0
13.............	7	0	0	9	0	0	16	0	0
14.............	7	0	0	12	0	0	19	0	0
	115	31	31	193	32	41	308	53	72

The most evident change in this series came suddenly on the eighth day. In the celloidin sac no typhoids were found on this date, or at any subsequent time, and in the parchment sac only one typhoid colony appeared on the eighth day, and none thereafter.

The results of these three foregoing series (see Summary, Table 19) show some variation in the longevity of the typhoid organism, but not more than was observed in the earlier series, where the exposure was in lake water alone. In general the typhoid bacillus persisted in these cases for about seven or eight days, with an occasional instance where vitality was prolonged for 10 or 11 days. These results stand in striking contrast to those obtained in the sewage series. This variation in longevity is brought out under optimum conditions in the case of two of the series that were run under conditions identical except as to the nature of the liquid in the sac.

Series VI and IX were immersed in the same sewage and run at the same time. Series VI contained sacs filled with lake water, while in Series IX the sacs were filled with sewage. The sewage series also received much the heavier seeding. As to results, the typhoid organism died in the sewage sacs in from three to five days, while in the sac filled with lake water, but immersed in same stream of sewage, it lived seven to eight days.

To permit of more ready comparison of results, reference can be made to Table 19, in which is summarized the results of all of the experiments made on the sewage series and those in which sacs were filled with lake water and immersed in sewage.

TABLE 19.

SUMMARY OF EXPERIMENTS WHERE EXPOSURE WAS MADE IN SEWAGE SACS, ALSO IN LAKE WATER SACS IMMERSED IN FLOWING SEWAGE.

	SERIES	SAC NO.	KIND OF SAC	DAYS															TOTAL NO. COL. FISHED	NO. PROV'N TYPHOID COL.
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Sewage sacs	V	12	Agar......	+	+	+		+1	+1	o	o	o				o				
		13	Parchment.		+	+	+	+1	+1	o	o	o				o				
		14	Celloidin . .		+	+	+1	+1	+	o	o	o				o			409	71
	VI	15	Parchment.	+	+	+	+	o	o	o		o		o			o	o		
		16	Celloidin . .		+	+	+	o	+	o		o		o			o	o	259	59
Lake water sacs	VII	17	Parchment.	+	+	+	o	+	+		+		o		o		o	o		
		18	Parchment.	+	+		+		o		o	o	o	o				o		
		19	Celloidin . .	+	+	+	+	+	+		o		+1		o		o	o		
		20	Celloidin . .	+	+	+	+	+		+		+		o	o		o	o		
		21	Agar......	+	+	+	+	+	+		o		o		o		o	o		
		22	Agar	+	+	o	+	+		+		+		o	+1		o	o	310	96
	VIII	23	Parchment.		o	o		+		+				+	o	o	o	o		
		24	Celloidin . .	+		o		+		leak discovered									169	36
	IX	25	Celloidin . .	+		+		o		+	+	o		o			o	o		
		26	Parchment.	+		+		+		+	+	+1					o	o	308	72
																			1,455	334

The evident conclusion which these data support is that the results obtained in the three series (VII–IX) in which the sacs were filled with typhoid-infected lake water and immersed in sewage are much more nearly in accord with the series exposed to running water (I–IV) than to those subjected to the direct action of sewage (V–VI). This would seem to indicate that the more destructive influence of sewage was not exerted unless the typhoid organism was in intimate contact with the sewage bacteria themselves.

Series X. One more possible combination existed, which was tried in order to test all aspects of the question at issue. Sacs filled with water had been immersed in flowing water; those filled with sewage had been placed in a sewage bath; and others filled with water had also been subjected to the influence of flowing sewage. The remaining combination of sewage-filled sac immersed in flowing water was therefore tried. In this case only a single sac was used (parchment). It was heavily inoculated with typhoid, 4,350,000 organisms per c.c. of a 24 hour culture.

A rapid death of the introduced germ occurred in this sac in the course of a few days. The latest recovery was on the fifth day, although even prior to this it had been greatly reduced in numbers. This result is practically the same as the sewage sacs immersed in

TABLE 20.

LONGEVITY OF B. TYPHOSUS IN PARCHMENT SAC FILLED WITH SEWAGE AND IMMERSED IN WATER.

DAYS	SAC 27 (PARCHMENT)		
	No. Colonies Fished	No. Pres. Typhoid	No. Proven Typhoid
0	20	10	19
1	2	2	2
2	30	6	6
3	18	2	2
4	12	0	0
5	12	1	1
6	4	0	0
8	0	0	0
10	5	0	0
13	17	0	0
14	9	0	0
	129	30	30

sewage. Although undoubtedly considerable diffusion of soluble substances would occur in this case, no material variation in the longevity of the pathogenic organism was observed. This is in accord with all of the previous results, and indicates that the longevity of B. typhosus is diminished when the organism is in direct contact with sewage bacteria.

In Table 21 are compiled the results of all experiments, expressed in days. This signifies the maximum period of time in each experiment during which the typhoid bacillus could be recovered. For findings other than the end results, comparative summaries may be found in Tables 10 and 19.

TABLE 21.

SUMMARY OF RESULTS.

B. TYPHOSUS EXPOSED TO	SACS IMMERSED IN	SERIES No.	LONGEVITY OF TYPHOID (IN DAYS)			
			Agar	Celloidin	Parchm't	Glass
Lake water	Lake Water	1	8, 8	8, 10		
		2	8	8		
		3		6	6, 8	
		4		10		13
Sewage	Sewage	5	5	5	5	
		6		5	3	
Lake water	Sewage	7	5	8, 9	3, 7	
		8		?	10	
		9		7	8	
Sewage	Lake water	10			5	

CONCLUSIONS.

1. Three types of permeable sacs (celloidin, parchment, and agar films) were employed to hold the typhoid organism imprisoned while it was exposed to the influence of water and sewage bacteria.

2. Tests made with chlorides, sugar, and peptone indicate that these sacs were readily permeable, while other tests demonstrated that they were wholly germ-tight.

3. In four series of examinations where B. typhosus was exposed to the action of flowing lake water (Mendota), the longevity of the organism ranged from 8 to 10 days, agreeing quite closely with the experiments previously reported on Lake Michigan water under similar experimental conditions.

4. Where B. typhosus was exposed directly to the action of sewage bacteria, its longevity was greatly diminished, three to five days being the longest time for which the organism could be recovered.

5. When the typhoid organism was exposed to the diffusible products of sewage bacteria, and not to the direct action of the organisms themselves, as was the case when the typhoid infected lake water-filled-sacs were exposed to flowing sewage, the longevity of the inoculated pathogenic form was increased. The results, although more variable than in either of the preceding cases, agreed more closely with those obtained in the lake-water series than with those exposed to the action of sewage itself. This would seem to indicate that the direct contact with sewage organisms was more detrimental to the vitality of the typhoid organism than the diffusible by-products of sewage forms.

6. Where typhoid-infected sewage-filled sacs were exposed to water, the longevity of B. typhosus was the same (five days) as where the bathing liquid was sewage. This again seems to indicate that the question of longevity is more dependent upon the actual contact with sewage types than it is upon contact with by-products capable of diffusion through these permeable membranes.

7. The uniformity noted in the results obtained in this investigation, and their confirmation of the work of the preceding year on the waters of Lake Michigan and the Chicago Drainage Canal would now seem to warrant the definite conclusion that the longevity of the typhoid bacillus in waters is materially affected by the germ content of its surroundings. In waters highly polluted with saprophytic bacteria, such as is the case in sewage, this disease organism is unable to survive for more than a few days (three to five in the experiments here described), a period of time materially shorter than that which is noted in normally unpolluted waters.

ON THE RELATION BETWEEN OXYGEN IN WATER AND THE LONGEVITY OF THE TYPHOID BACILLUS.

GEORGE C. WHIPPLE AND ANDREW MAYER, JR.

THERE are many factors which affect the longevity of the typhoid bacillus in water. Experiments which we have made at various times during the last two years indicate that the presence of dissolved oxygen is one of the most important. Comparative experiments made by putting typhoid bacilli into two portions of water and maintaining one under anaerobic conditions, while the other remained oxygenated, indicated that the bacilli preserved their vitality for a much longer period when oxygen was present. This was found to be true also of the colon bacillus.

The experiments were varied in a number of ways, as shown by the following tables. The culture of typhoid bacillus, used in all the experiments, was obtained from Dr. Ezra Wilson of the Hoagland Laboratory. Before using it, it was submitted to preliminary cultivation in broth for 24 hours at 37° C. From the third generation in broth an agar streak culture was made, which was used in subsequent inoculations.

EXPERIMENT 1.*

Brooklyn tap water was filtered through a Berkefeld filter, and 19 c.c. were put into test tubes and sterilized. To each of these tubes was then added 1 c.c. of a suspension of typhoid bacilli in water. Another series of tubes was prepared in a similar way, but the water was boiled to expel the dissolved oxygen. The tubes of this series were inoculated with typhoid bacilli as in the previous case.

The first set of tubes was placed in the 20° incubator; the second was also placed in the incubator, but was kept in a Novy jar in an atmosphere of hydrogen. After various periods of incubation, one tube of each set was withdrawn and samples were plated. The following table represents the results obtained:

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* Made with the assistance of Mr. Luther R. Sawin, Bacteriologist, Dept. of Water Supply, Gas, and Electricity.

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN HYDROGEN	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
April 13	0	600,000	100.0	600,000	100.0
April 15	2	455,000	76.0	2,400	0.4
April 17	4	190,000	32.0	25	0.004
April 21	8	120,000	20.0	0	0.0
April 25	12	67,000	11.0	0	0.0
May 1	18	25,000	4.2	0	0.0
May 9	26	9,250	1.5	0	0.0
May 16	33	2,150	0.6	0	0.0
May 23	40	132	0.02	0	0.0
May 30	47	6	0.001	0	0.0
June 6	54	0	0.000	0	0.0

EXPERIMENT 2.

The second experiment differed slightly from the first one. Sterilized distilled water was used instead of tap water, 150 c.c. was the quantity taken, and flasks were employed instead of test tubes. The water was not boiled. Carbonic acid was used in the Novy jar instead of hydrogen. The results obtained are given in the following table:

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN CARBONIC ACID	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
July 18	0	400,000	100.0	400,000	100.0
July 20	2	265,000	66.0	110,000	27.5
July 22	4	......	....	1,500	0.38
July 26	8	50,000	12.5	0	0.0

EXPERIMENT 3.

The experiment was made as above described except that an atmosphere of nitrogen, obtained by using pyrogallic acid and caustic potash, was used for the anaerobic flasks. The results are shown in the table following:

Period of Time	Kept in Air Number per c.c.	Kept in Nitrogen Number per c.c.
0 hours	3,120,000	3,120,000
24 "	No	0
48 "	record	0
72 "	kept	0

EXPERIMENT 4.

This experiment was precisely like No. 3. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
0 hours	880,000	100	1,390,000	100
24 "	710,000	81	0	0

EXPERIMENT 5.

This experiment was like No. 3 except that no control culture was made in air. The results were as follows:

PERIOD OF TIME	TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number
0 hours	164,000	100.0
2 "	36,000	23.0
4 "	12,240	7.5
24 "	0	0.0

EXPERIMENT 6.

This experiment differed from the preceding in that 1 c.c. of the broth culture was used to inoculate the water. This apparently produced a culture medium in which the bacilli could multiply. It was observed, however, that after multiplication had taken place the numbers fell off more rapidly in the anaerobic jar. The results were as follows:

Period of Time	Tubes Kept in Air Number per c.c.	Tubes Kept in Nitrogen Number per c.c.
0 hours	1,500,000	1,500,000
24 "	....	3,000,000
7 days	7,200,000	4,000,000
21 "	5,000,000	550,000

EXPERIMENT 7.

This experiment was conducted like the others, but B. coli was used instead of B. typhosus. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
Jan. 1–28, 1905				
0 hours	3,400,000	100	3,400,000	100.0
12 "	....	....	648,000	19.0
24 "	620,000	18	288,000	8.5
72 "	....	..	10,500	0.3

If it be true, as indicated by these experiments, that the presence of dissolved oxygen in water causes typhoid fever bacilli to maintain their vitality for longer periods of time than when no oxygen is present, this throws some light on the results of various observations which have been made by other investigators. For instance, Jordan, Russell, and Zeit[1] found by their experiments on the waters of the Chicago Drainage Canal and the Illinois River that typhoid bacilli disappeared more quickly in polluted water than in relatively pure water. Among other reasons for explaining this fact, it is quite possible that the absence of oxygen in the polluted waters may have been an important influence. There seems to be some evidence that typhoid fever germs maintain their vitality more readily in the winter season than during the summer. It is worth noting in this connection that cold water contains much larger amounts of dissolved oxygen than does warm water. The effect of the septic tank on the longevity of pathogenic bacteria also involves the relation of oxygen

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[1] *Jour. Infect. Dis.*, 1904, 1, p. 641.

THE RELATIVE APPLICABILITY OF CURRENT METHODS FOR THE DETERMINATION OF PUTRESCIBILITY IN SEWAGE EFFLUENTS.

George A. Johnson, William R. Copeland, and A. Elliott Kimberly.

Less attention was formerly paid to the question of the putrescibility of sewage effluents than is the case at the present time. For the most part the purification processes then embraced either broad irrigation fields and intermittent sand filtration, which normally yield effluents of a high degree of purity, or chemical precipitations which remove only about one-half of the total organic matter. To-day there are many sewage problems in which purification require to be carried apparently only to the extent of obtaining a non-putrescible effluent. For this purpose coarse-grain filters have a wide field of usefulness, and they also are a factor to be considered as an intermediate treatment in those sections where porous, sandy soil is not available naturally, and where thorough purification is needed.

Contact filters, or sprinkling filters of broken stone, do not, of course, effect as high a degree of purification at their best as do filters of fine-grain material, and when unskilfully operated may yield effluents containing sufficient unstable organic matter to render them putrescible. To obtain the most satisfactory results from such processes, frequent data are required regarding the putrescibility of the effluent. The great need, therefore, as is universally recognized, is for a test, whereby a determination of the character of the effluent, so far as relates to its putrescibility, may be speedily made, and thus enable the results to serve as a direct guide in operating the plant.

The putrescibility tests now in general use, from a practical standpoint, possess a common weakness in that nearly all, if indeed not all, are based upon a method whereby the samples of the effluent require incubation for several days before the result can be definitely known. So far as they are of assistance to the operators of sewage works, the results of such tests are of value only as matters of record, to serve as a general guide in future operating procedure.

Numerous attempts have been made to fix certain arbitrary standards of purity of sewage effluents by prescribing that an effluent shall not contain more than so much organic nitrogen, or oxygen absorbed, thus (the data expressed in parts per million)—the Rivers Pollution Commission require an effluent to be below organic carbon 20, organic nitrogen 3; Thames Conservancy, organic carbon 30, organic nitrogen 11; Derbyshire County Council, albuminoid ammonia 1, oxygen absorbed 10 (4 hours' test); Ribble Board, albuminoid ammonia 1, oxygen absorbed 20 (4 hours' test); Mersey and Irwell, albuminoid ammonia 1.4, oxygen consumed 14.[3] At this time, however, there is an almost unanimous feeling against even the limited applicability of such standards. Albuminoid ammonia, organic nitrogen, and oxygen consumed indicate, not the exact proportion of putrescible matter in a sample, but rather, generally speaking, only a component part of these substances. Nitrogen, as determined by these tests, may be derived both from stable and unstable matters; organic carbon, as indicated by the oxygen consumed tests, may be represented not only by putrescible carbonaceous organic matter, but also by certain non putrescible substances requiring oxygen for their oxidation.

The putrescibility of a sewage effluent, as is quite generally recognized at this time, is intimately related to its composition with respect to its relative content of stable and unstable organic matter, readily oxidizable mineral substances, and the relative abundance of available oxygen present. For this reason it is certain that the mere knowledge of the amounts of putrescible organic matter present in a sample would be insufficient evidence upon which to base an opinion of the putrescibility of the sample. A knowledge of the amount of available oxygen present to counteract the putrescible tendencies of the sample is required. With a fairly accurate knowledge of the oxygen consumed by, and the oxygen available in, a sample, it is probable that in some cases at least it may be possible to fix arbitrary standards of purity of sewage effluents, so far as their putrescibility is concerned.

INCUBATOR TESTS FOR THE DETERMINATION OF PUTRESCIBILITY.

Brief historical résumé. — The first recorded test for the character of sewage polluted water, so far as we are aware, was proposed in 1870 by Heisch,[1] who noted the fouling and growths produced when the sample, mixed with cane sugar, was exposed to sunlight. In 1884, Dupré[2] stated that when sewage polluted water was kept for 10 days out of contact with the air, a more or less complete absorption of the dissolved oxygen would take place, and that by a determination of the dissolved oxygen before and after incubation, an idea might be obtained regarding the amount of organic matter present. This process, as pointed out by Rideal,[3] aimed at determining the number of organisms present in the sample, rather than the amount of putrescible compounds contained therein.

Scudder,[4] chemist to the Mersey and Irwell Joint Committee, introduced in 1895 the incubator test, which, subject to a number of modifications, is in more or less general use at the present time. At the inception of this test, the procedure simply consisted in completely filling a bottle with the sample, stoppering tightly, and incubating for a few days at summer temperature. Later[5] Scudder refined the method by including in the technique the determination of the oxygen absorbed in cold acid permanganate in three minutes, before and after an incubation period of from five to six days at 75° F. This method was also used by Clowes[6] in the London experiments, and by Fowler[7] at Manchester. In the early Manchester experiments[8] this test was modified by the use of a period of incubation of from six to seven days at 80° F. Scudder[9] later included the determination of dissolved oxygen and nitrates, before and after incubation, since he found that effluents which did not show an increase in the amount of oxygen absorbed from permanganate would also contain after incubation residual amounts of dissolved oxygen and nitrates.

Ross[10] incubates at 80° F. for seven days, and further estimates the degree of putrescibility of the samples by determining the oxygen absorbed from permanganate in 15 minutes and four hours, respectively, at a temperature of 80° F. This method is also used by Wilkinson,[11] at the Oldham Sewage Works. Stoddart's[12] modification of the incubator test aimed to place the determination of the odor of putrescible samples upon a more severe basis, by making a simple quantitative test for hydrogen sulphide. Clark[13] incubates the samples at 80° F. for two to five days, and determines the oxygen consumed from acid permanganate during a two-minute boiling period, before and after incubation.

Attempts have been made in a number of instances to place the incubator test on a more practical basis, by imitating in the laboratory conditions such as obtain when the effluent is discharged into the watercourse. This was effected by diluting the sample with varying volumes of river water, corresponding to the dilution obtainable in practice, following which the sample was incubated at summer temperature for a number of days. Thus Adeney[14] suggests the dilution of the sample with river water before incubation. At Manchester,[15] it has been the practice for some time to incubate mixtures of the effluents and canal water. Clark[16] mixes equal volumes of effluent with tap water, and determines the amount of dissolved oxygen before and after incubation for five days at 80° F. In conjunction with this test he uses the two-minute oxygen consumed test already referred to, together with determinations of the amount of nitrites and nitrates present before and after incubation. Dunbar and Thumm[17] incubate the sample for one week in closed flasks at 68° F., note the formation of odors, and determine the presence of hydrogen sulphide with lead acetate paper.

The above is a necessarily somewhat incomplete digest of published literature bearing upon the incubator test for the determination of putrescibility. As is generally recognized, all of these tests, while yielding results of definite value, fall short of the most practical requirements, since the results obtained by these methods are not immediately available for the guidance of the sewage works operator.

Results of the studies at Columbus on the incubator test. — In a critical study of the incubator test and its various modifications at the Columbus Sewage Testing Station, we first turned our attention to a means of reducing the period of incubation. By adopting a temperature of 37° C., we were able in a majority of cases to obtain the same results as when, at lower temperatures, periods of incubation of several days were employed. Many determinations indicated that if odors did not develop after 24 hours at 37° C., they would also not be apparent after five days at 27° C. The relation between the period and the temperature of incubation in effecting putrefactive changes is clearly shown in the following table:

TABLE I.

EFFECT OF TEMPERATURE AND PERIOD OF INCUBATION ON THE RAPIDITY OF THE DEVELOPMENT OF PUTRESCENCE IN SEWAGE EFFLUENTS.

Character of Device	Coarse-Grain Filter		High Rate Sand Filter	
Temperature of incubation, deg. C.	27	37	27	37
Period of incubation, days	5	1	5	1
Number of samples which gave positive results	117	108*	21	18*
Per cent which positive results obtained at 37° C. were of those obtained at 27° C.	92		86	

*The balance of the samples incubated at 37° C. gave positive results at the end of 48 hours.

Our studies of the incubator test at Columbus, as outlined above, have shown that the period of incubation may be advisedly reduced to 24 hours, provided a temperature of incubation of 37° C. (98.5° F.) be used, since about 90 per cent of the samples incubated at 48 hours at this temperature developed odors of putrefaction in 24 hours.

SPECIAL CHEMICAL TESTS FOR DETERMINING THE PRESENCE OF PUTRESCIBLE COMPOUNDS IN SEWAGE EFFLUENTS.

Attempts have been made to determine by direct chemical tests the putrescibility of sewage effluents. Spitta[18] suggests that by adding to the sample enough methylene blue to impart a faint color, putrescible samples will quickly and completely discharge the blue color when incubated for a few hours at a temperature of 72° to 79° F. Fowler[19] has suggested that this test has quantitative possibilities which have yet to be worked out.

In the Columbus studies, consideration was given to this test, as well as to others, whereby the presence of albuminous and proteid matters might be directly determined. From a study of the methylene blue test, we have found that oftentimes there would be present substances other than putrescible organic matters, such as sulphide of iron and hydrogen sulphide, which would immediately discharge the blue color, before the putrescible matters were able to act.

The other tests studied in this connection included the biuret reaction[20] and the use of Millon's reagent,[21] whereby it was thought that the presence of undecomposed albuminous substances could be detected. After an extended trial it was found that these tests did not distinguish between putrescible and non-putrescible substances; chiefly, it is thought, because putrescible substances are not confined wholly to the albuminoid class.

In addition to these studies, attempts were made to determine the putrescibility of an effluent by measuring its propensity to absorb oxygen from chemical oxidizing agents. First among these, iodine and hydrogen peroxide were studied. While it is true that, in connection with the use of all chemical oxidizing agents, certain complications arise in the presence of inorganic reducing agents, iodine and hydrogen peroxide are reagents of such high sensibility, that there are many inherent practical difficulties encountered in their use, which bar them from serious consideration among tests for determining the propensity of the effluent to consume oxygen.

In the further work at Columbus, a method was sought for the determination of putrescibility, which would correlate the results of the regular chemical analysis and the putrescible properties of the effluent. The direction in which the solution of the question seemed to lie, as has been previously noted, was in the balance between the oxygen available to offset the putrefactive tendencies of the effluent, and the actual amount of oxygen consumed in effecting this result. It was considered that in a certain degree the amount of oxygen consumed could be referred to the regular "oxygen consumed" values from permanganate. Where the amount of available oxygen exceeded the amount of oxygen consumed, it appeared certain that such conditions favored the ultimate complete oxidation of the unstable matters without accompanying nuisances.

In order to obtain information on the relation between the results obtained by the various "oxygen consumed" tests, coincident with the putrescibility of the sample as referred to the odor developed after incubation in a tightly stoppered bottle for 24 hours at 37° C., the several tests were applied to a large number of samples collected from various sources. The modifications in the oxygen consumed test which were used in this comparative study were as follows: (*a*) The instantaneous oxidation by permanganate in a cold acid solution;[22] (*b*) Oxidation by permanganate during a three-minute period of digestion in a cold acid solution;[23] (*c*) Ditto in a 15-minute period of digestion;[24] (*d*) Oxidation by permanganate during a period of digestion of 30 minutes in boiling water;[25] (*e*) Oxidation by permanganate at boiling temperature during a five-minute period.[26] Duplicate samples were also incubated, as above described, and their putrescibility determined by the odor test. The results, which are given in the following table, indicate that the relative amounts of "oxygen consumed," as shown by the modified methods according to which the more rapid determinations are made, are naturally greater in the putrescible than in the non-putrescible samples. The more prolonged and higher temperature tests simply serve to emphasize these differences.

TABLE 2.

Comparison of Oxygen Consumed Results Obtained by Different Methods of Analysis in Putrescible and Non-Putrescible Samples.

Method	Time of Contact (Minutes)	Temperature, Deg. F.	Oxygen Consumed Parts per Million		Relation of Results by Other Methods to Those Obtained by the Boston Method	
			Putrescible Samples *a*	Non-Putrescible Samples *b*	*a*	*b*
Immediate	1	80	3.5	1.5	0.17	0.21
English	3	80	4.2	1.4	0.20	0.20
English	15	80	9.7	2.5	0.46	0.35
Boston	5	Boiling	20.9	7.2	1.00	1.00
Palmer	30	"	44.7	12.0	2.13	1.67

RELATION OF THE COMPOSITION OF A SEWAGE EFFLUENT TO ITS STABILITY.

Unstable organic and inorganic matter. — Putrescibility may be due to the presence of complex bodies of either animal or vegetable origin, depending entirely upon the character of the raw sewage. The relative amounts of these two classes of organic matter are roughly indicated by the amounts of organic nitrogen, and "oxygen consumed," respectively. Since the same amounts of nitrogen or oxygen consumed may be present in one case in a crude sewage, and in another in a stable effluent, it is clear, as pointed out by Dunbar and Thumm,[27] that it is the relative, and not the absolute, amount of organic matter in sewage before and after purification, that is to be taken as the criterion for the consideration of questions relating to putrescibility.

The breaking-down by hydrolysis of the complex, highly organized bodies containing sulphur gives rise to the formation of considerable amounts of sulphureted hydrogen. Sulphur in this form rarely occurs in sewage effluents, however, since the iron in the crude sewage, or in the filtering material, combines with the sulphureted hydrogen to a sulphide, which is ultimately oxidized to sulphate in effluents which are normally stable. In fact, the absence of sulphide of iron in the effluent[28] may be taken as an indication of the adequacy of the aeration facilities within the filter; and further, perhaps of more moment for the putrescibility question, sulphide of iron will not develop in an effluent when stored, provided the conversion to a stable form of the organic matter therein has been sufficiently completed in the filter. The putrefactive tendencies of a sewage effluent thus refer to unstable organic bodies which are generally found to be coincident with the presence, or the subsequent formation, of sulphide of iron.

THE AVAILABILITY OF OXYGEN OF DIFFERENT KINDS AS FOUND IN SEWAGE EFFLUENTS.

In the case of some of the rapid processes, a very considerable proportion of the purification effected by rapid filters of coarse material may be said to take place under anaerobic conditions, and through agencies in which oxygen is not directly concerned. But, as has already been mentioned, the great majority of causes underlying putrescible conditions in sewage effluents refer to a supply of oxygen within the filter insufficient to effect the changes which bring about stable conditions in the effluent.

Availability of gaseous oxygen and oxygen combined with nitrogen. — In a sewage effluent there are two sources of oxygen, which the studies of a number of workers besides ourselves have shown to be available for the protection of the effluent against putrefaction. These are dissolved gaseous oxygen, and the oxygenated compounds of nitrogen, nitrogen pentoxide (N_2O_5), and nitrogen trioxide (N_2O_3), commonly spoken of as nitrate and nitrite oxygen, respectively. Rideal[29] says that the "available oxygen" is that present as nitrate and nitrite, and that the amount in a satisfactory effluent is quite sufficient to overcome putrefaction, without the aid of the dissolved oxygen in the stream into which the effluent is discharged. By adding certain amounts of sodium nitrate to clarified sewage, Adeney and Scott-Moncrief[30] take advantage of the availability of oxygen from that source. Sewage so treated, after retention in a tank for several hours, may be discharged therefrom in a stable condition. During the experiments at Manchester,[31] the effect of mixing a nitrated and a crude tank effluent was studied as a means of reducing the required acreage of the purification works. The result of these studies showed that, within certain limits, a considerable volume of crude tank effluent could be rendered stable in this manner. In experiments in which equal volumes of tap water and putrescible sewage effluent were kept at summer temperature for several days, Clark[32] showed a marked diminution in the initial amounts of dissolved oxygen and nitrate oxygen. In non-putrescible samples, treated similarly, no such pronounced reduction in the available oxygen took place, and an appreciable amount of residual oxygen was always noted. Fowler[33] also affirms that a well-nitrated effluent is protected against putrefactive tendencies. The well-known researches by Gayon and Dupetit[34] show that marked decomposition of unstable organic compounds is effected by the reduction of nitrates by bacteria. Similar observations have been made by numerous other observers.

Availability of other sources of oxygen. — There are a number of oxygenated compounds in sewage effluents, besides those already discussed, which require a passing comment regarding the availability of oxygen from such sources for overcoming putrescible conditions. Sulphates and oxide of iron are among those which particularly suggest themselves. The question arises, however, whether we are to regard oxygen, chemically combined in such stable atomic aggregations, as available under the reduction forces at work when a but partially purified sewage undergoes putrefactive change. While the bacterial reduction of sulphate to sulphide, and of ferric oxide to its ferrous state, has been noted by other observers, the conditions were somewhat different from those encountered in studies relating to the putrescibility of sewage effluents. Clark[41] is of the opinion that sulphates in sewage will undergo a considerable decomposition under septic conditions, the extent of their reduction in a septic tank, in which a period of flow of about 24 hours was maintained, being shown as about 7 per cent. Fuller[42] has shown that in the sediment deposited from Ohio River water, prior to the process of the decomposition of the organic matter contained therein, it is probable that there took place a reduction of the oxygen present in the mineral compounds, such as sulphates and nitrates, and a reduction of ferric to ferrous iron.

During the studies on the putrescibility question made at the Columbus Sewage Testing Station, such question as these have been given a careful consideration. For the purpose of learning whether any considerable reduction of the sulphates, which the Columbus sewage normally contains, took place under the active reducing actions present in septic tanks, the influent and the effluent of two of the septic tanks in operation at the testing station were sampled at half-hourly and hourly intervals, respectively, for a period of one week during the month of May. At the end of that time the various portions were mixed and examined for sulphates. The results of this experiment are presented in Table 3 and show conclusively that sulphates (SO_4), present in the applied sewage to the extent of 200 parts per million, did not suffer any appreciable reduction when exposed fcr 16 and 24 hours, respectively, to the reducing action incidental to the septic process under local conditions.

While, as Fuller[42] has pointed out, it is probable that oxide of iron will yield up its oxygen under the conditions stated, its reduction implies highly putrescent conditions such as would not obtain in an effluent of ultimate stability. It seems clear, therefore, that in overcoming putrescible tendencies in a sewage effluent, only gaseous oxygen and oxygen combined with nitrogen can be considered as available sources of oxygen in effecting bacterial oxidation to a condition of ultimate stability. Further, as already noted, fixed

TABLE 3.

TABLE SHOWING THE EFFECT OF SEPTIC ACTION UPON THE SULPHATES IN CRUDE SEWAGE.

DEVICE	PARTS PER MILLION SO_4		
	1	2	Average
Crude sewage	208	208	208
16 Hour septic tank	203	205	204
24 Hour septic tank	201	...	201

NOTE. — Method of determining sulphates: In boiling acidified solution precipitated sulphate with barium chloride. Filtered through tarred Gooch crucible, ignited, and weighed.

standards referring to certain maximum permissible amounts of organic nitrogen, albuminoid ammonia, or oxygen consumed, cannot be taken as indications of such conditions.

METHODS FOR THE DETERMINATION OF PUTRESCIBILITY BASED UPON CURRENT CHEMICAL DATA.

With a thorough knowledge of all the factors associated with the causation of putrescibility, it would seem that the results of chemical analysis which, up to the present time and now, represent the extent to which the differentiation of the several constituents in the effluent may be carried, should furnish also information as to the putrescible properties of a given sample. Considering introspectively the nature of the conditions under which putrescibility takes place, and studying carefully also the factors which are instrumental in preventing the putrescent reorganization of unstable organic matter, we are forced, like others, to the conclusion that the putrescibility factor narrows itself down to the amount of oxygen available for aerobic decomposition.

The oxygen consumed results, as obtained by the standard five-minute boiling method,[26] measure fairly accurately the amount of oxygen consumed in the oxidation of the unstable matter present in the sample. Since the reactions, as a result of which the ultimate reorganization of crude organic matter takes place under aerobic conditions, are subject to the uncertainties incidental to the action of bacteria, it would seem inadmissible to infer that the amount of oxygen necessary to complete the oxidation of the putrescible organic constituents of the sewage effluent, as indicated by chemical oxidizing agents, would be the same as that necessary for the accomplishment of the oxidation of such matter by bacteria. It seems certain, therefore, that the most probable direction in which would lie the satisfactory interpretation of "oxygen consumed" results, with respect to the premises of putrescibility, refers to the establishment of a relation between the oxygen absorbed from permanganate and its bacterial equivalent.

The oxygen available in a sewage effluent to offset its putrescible tendencies, as has already been pointed out, is the amount of oxygen present in the sample in a dissolved gaseous state and that combined with nitrogen in the form of nitrate and nitrite. A measure of the putrescible tendencies of an effluent may be closely estimated from the amount of oxygen absorbed from permanganate during a three-minute digestion of the sample in a cold acid solution. This feature has been put forward many times before by other workers, and it is merely our desire at this time to record the fact that our work confirms the stability of their conclusions. We desire to emphasize the fact, however, that in some instances this may not be absolutely true, but for the majority of cases we believe that such is the case. Further, the results of our studies lead us to feel that under average conditions there is an intimate relationship existing between the three-minute cold test,[23] and the five-minute boil test.[26] In Table 2 it was clearly shown that the former value was one-fifth of the latter.

It is the available oxygen contained in the effluent in the form of dissolved oxygen and oxygen combined as nitrates and nitrites, which may be said to serve as a protecting agent in preventing the establishment of putrescent conditions. Our studies indicate that the existing relation between the oxygen absorbed from permanganate in cold acid solutions in a period of three minutes — or a similar "oxygen consumed" value obtained by dividing the result of the five-minute boil test by five — and the available oxygen which the effluent contains, may furnish a satisfactory means whereby the oxygen required and available, respectively, may be placed upon a comparable basis.

RELATIVE AVAILABILITY OF DISSOLVED OXYGEN AND OXYGEN COMBINED WITH NITROGEN IN THE NEUTRALIZATION OF PUTRESCIBLE TENDENCIES IN SEWAGE EFFLUENTS.

In our studies of the putrescible qualities of sewage effluents, and those factors which serve to prevent putrescence, certain facts were brought out with considerable distinctness. We desired to learn if a fairly definite relationship existed between the consumed and the available oxygen, as indicated by the results obtained through the medium of certain "oxygen consumed" methods, and by the amounts of oxygen contained in the effluent which could be properly considered as available for the prevention of putrescent conditions. A large number of tests were made to determine the "oxygen consumed" by the three-minute cold and the five-minute boil methods, respectively, together with the amount of available oxygen contained in the effluents of the various devices under study at the Sewage Testing Station. The results of these studies brought out the fact that there does exist a more or less definite relation between the amount of oxygen consumed, as determined by chemical tests, the dissolved oxygen, and that available through bacterial action from the nitrates and nitrites which the effluent contains. In the course of these studies, carried on side by side with incubator tests, we were inclined to believe that in cases where the oxygen consumed, as shown by the chemical tests described above, was, before incubation, less than the amount of available oxygen computed from the amount of dissolved oxygen and from those amounts available from nitrites and nitrates, respectively, such an effluent would not putrefy on incubation; and such, in the majority of cases, has turned out to be the case.

To place such deductions as these on a stable basis, we realize that a definite statement is required as to the respective coefficient of availability of oxygen dissolved in the effluent in the free gaseous form, and that present in combination with nitrogen in the form of nitrate and nitrite. In this connection we have already noted that sprinkling filters yield at times a putrescible effluent which, while not well nitrified, may still contain amounts of dissolved oxygen sufficiently great to prevent the establishment of putrescent conditions, if the availability coefficient of dissolved oxygen were unity. That the reverse has been found to obtain in many instances is shown by the results presented in the next table. These data are representative of many similar cases, where, under the same conditions with respect to the content of the effluent in organic nitrogen and oxygen consumed, the effluent was clearly putrescent after incubation. In these cases, while the nitrate and nitrite oxygen were present in low quantity, the amount of gaseous oxygen dissolved in the effluent was high. Dissolved oxygen plays such an important part in the disposal of sewage in flowing streams by preventing putrescent conditions therein that such results as these, in the absence of more extended evidence, are clearly to be regarded as abnormal. It is therefore the desire of the writers merely to suggest the possibility that such conditions may arise in the case of sprinkling filter effluents with the thought that perhaps further investigation under a different set of conditions may satisfactorily explain them.

TABLE 5.

AVAILABILITY OF DISSOLVED OXYGEN.

PARTS PER MILLION					PUTRESCIBILITY TEST BY ODOR AFTER INCUBATION	OXYGEN CONSUMED (5 MIN. BOIL÷5) WAS TO OXYGEN AVAILABLE FROM:		
Oxygen Consumed			Available Oxygen					
5 Min. Boil÷5	3 Min Cold	Dissolved	Combined with Nitrogen	Total		Dissolved	Combined with Nitrogen	Total
7.6	6.5	7.1	4.1	11.2	+	1:0.93	1:0.54	1:1.47
6.6	6.3	3.5	5.2	8.7	+	0.53	0.79	1.32
6.0	6.0	5.2	2.4	7.6	+	0.87	0.40	1.27
5.6	5.8	5.7	1.6	7.3	+	1.02	0.29	1.31
6.0	5.5	7.3	1.5	8.8	+	1.22	0.25	1.47
5.2	5.7	6.6	3.1	9.7	+	1.27	0.60	1.87
5.8	5.5	8.3	2.0	10.3	+	1.43	0.35	1.78
5.4	6.0	8.4	2.5	10.9	+	1.56	0.46	2.02
5.0	5.5	8.3	2.7	11.0	?	1.66	0.54	2.20

In the following table the amount of oxygen consumed is represented both by the results of the five-minute boil method, and the three-minute cold method. In the computations of the amount of consumed oxygen the corrected results of the five-minute boil method are used, for the reason that it appears to us a somewhat more reliable method for indirectly measuring the amount of oxygen consumed by the easily oxidizable matter in a sewage effluent. The three-minute test, to our thought, possesses a considerable weakness in that it emphasizes disproportionately the value of inorganic compounds of ready oxidizability. Further, the technique entailed by this method is somewhat more delicate than that employed in the five-minute boil method, and subject to somewhat greater possibilities of inaccuracy.

TABLE 6.

SELECTED RESULTS OF PUTRESCIBILITY TESTS ILLUSTRATIVE OF THE VARIABLE VALUE OF THE SEVERAL AVAILABLE FORMS OF OXYGEN.

PARTS PER MILLION					Putrescibility Test by Odor after Incubation	OXYGEN CONSUMED (5 MIN. BOIL ÷ 5) WAS TO OXYGEN AVAILABLE FROM:			Putrescent Conditions on Incubation Probably Due to the Inactivity of:
Oxygen Consumed		Available Oxygen							
5 Min. Boil	3 Min. Cold	Dissolved	Combined with Nitrogen	Total		Dissolved	Combined with Nitrogen	Total	
3.2	2.8	1.7	1.0	2.7	+	1 : 0.53	1 : 0.31	1 : 0.84	
4.2	3.9	7.4	7.0	14.4	?	1.76	1.67	3.43	
5.6	4.6	0.0	5.1	5.1	+	0.00	0.91	0.91	
7.2	9.2	6.2	0.9	7.1	+	0.86	0.12	0.98	
2.8	7.7	3.6	5.1	8.7	O	1.29	1.82	3.11	
3.2	4.5	0.1	2.5	2.6	+	0.03	0.78	0.81	
3.2	3.4	0.0	3.8	3.8	?	0.00	1.19	1.19	
5.0	3.8	7.7	2.7	10.4	?	1.54	0.54	2.08	
4.6	13.9	2.1	2.9	5.0	?	0.46	0.63	1.09	
4.2	6.8	1.6	4.9	6.5	?	0.38	1.17	1.55	
3.8	3.2	8.4	2.3	10.7	+	2.21	0.61	2.82	*
5.0	5.3	0.2	3.5	3.7	+	0.04	0.70	0.74	
9.8	3.7	6.4	8.0	14.4	+	0.65	0.82	1.47	*
4.0	3.4	7.9	6.8	14.7	?	1.97	1.70	3.67	
5.8	10.6	4.3	1.7	6.0	+	0.74	0.29	1.03	*
3.6	4.0	0.1	3.0	3.1	+	0.03	0.83	0.86	
7.2	8.8	5.3	5.5	10.8	+	0.74	0.76	1.50	*
4.4	4.2	6.7	4.1	10.8	?	1.52	0.93	2.45	
3.6	3.5	1.1	4.7	5.8	O	0.31	1.30	1.61	
5.2	5.0	3.6	1.4	5.0	?	0.69	0.27	0.96	
3.2	3.4	6.8	7.6	14.4	?	2.13	2.37	4.50	
3.2	3.6	0.0	5.8	5.8	O	0.00	1.81	1.81	
4.8	4.2	4.7	5.6	10.3	+	0.98	1.17	2.15	§
3.4	3.7	0.6	3.9	4.5	O	0.18	1.14	1.32	
2.2	2.8	0.1	2.1	2.2	?	0.05	0.95	1.00	
5.4	4.9	0.1	3.4	3.5	+	0.02	0.63	0.65	
5.4	6.3	6.3	2.5	8.8	O	1.17	0.46	1.63	
6.2	7.1	3.9	8.2	12.1	+	0.63	1.32	1.95	§
5.0	5.0	5.7	4.9	10.6	+	1.14	0.98	2.02	*
3.2	3.4	3.4	0.8	4.2	?	1.06	0.25	1.31	
4.2	3.6	2.5	3.2	5.7	O	0.60	0.76	1.35	
3.2	3.1	1.7	1.0	2.7	+	0.53	0.31	0.84	

NOTE. — + = Odor like H_2S, strong and lasting. ? = Ditto, but very faint and immediately disappearing. O = No offensive odor. * = Dissolved oxygen. § = Oxygen combined with N.

THE RAPID ESTIMATION OF THE PUTRESCIBILITY OF SEWAGE EFFLUENTS.

From a practical standpoint no information regarding the efficiency of a sewage filter is so promptly required as is knowledge of the putrescibility of the effluent. The methods in use at this time for the determination of this feature generally include a period of incubation of from five to seven or more days. There is an urgent need, however, as is recognized by all, of a method which will permit of at least an approximation of the putrescible character of the effluent of a filter, and which will yield information sufficiently accurate to make possible opportune changes in operating procedures whereby the character of the effluent may be improved at once, instead of several days after it has commenced to deteriorate. In the light of present knowledge on the subject, it would be unreasonable to expect a method to be found for this purpose which would, at all times and under all conditions, yield an absolutely correct result; but the results of our studies lead us to conclude that an idea of the putrescible character of a sewage effluent, sufficiently accurate for all ordinary purposes, may be secured in the space of an hour, or so, as follows:

Determination of putrescibility by computation from the chemical data. — The analytical data required include the "oxygen consumed" by the five-minute boil method, the nitrogen as nitrite and nitrate, respectively, and the dissolved oxygen. The oxygen consumed is estimated by dividing the "oxygen consumed" by five. This factor represents the coefficient whereby the "oxygen consumed" result may be converted to its bacterial equivalent, or the value indicating the amount of consumed oxygen. The amount of available oxygen is determined by converting the nitrogen, as nitrite and nitrate, respectively, to oxygen, by multiplying the nitrogen value of these two constituents by the factors 1.71 and 2.86, respectively, representing the relative amounts of oxygen combined with nitrogen in these two forms. The sum of the oxygen available from these nitrogen compounds, together with a somewhat uncertain proportion of the dissolved oxygen, represents the real active agent in the prevention of putrescent conditions in a sewage effluent.

With the data above outlined at hand, we feel that it is admissible to interpret the putrescibility of an effluent in the following manner:

First, when the consumed oxygen value is equal to or in excess of the amount of dissolved oxygen in the effluent, and no nitrates or nitrites are contained therein, the sample will putrefy.

Second, when the consumed oxygen value is equal to or slightly less than the amount of oxygen contained in the effluent in the form of nitrates, nitrites, and dissolved oxygen, the sample may or may not putrefy.

Third, when the consumed oxygen value is less than the oxygen contained in the effluent in the form of nitrates and nitrites, under ordinary circumstances the sample will not putrefy.

Such are the relations which our results indicate as existing between the constituents of chemical analysis and putrescibility. While we have found that such deductions, as outlined above, admitted of quite extended application to conditions obtaining at Columbus, we do not wish to be misunderstood as contending that these provisional criteria of putrescibility will prove to be of general applicability, under the great variety of conditions encountered in the practical control of sewage works. We feel, however, that the knowledge gained in these studies possesses considerable suggestive value, which may possibly serve as a basis for future studies along similar lines.

The chemical tests involved in the rapid estimation of putrescibility, as outlined above, are such that the services of a competent analyst will be required in connection with the sewage works where such tests may prove to be applicable. In small works, where the expense of such a man might be considered as prohibitive, the incubator tests may be employed to afford the desired information. In this connection we desire to call attention to the feasibility of employing a period of incubation of 24 to 48 hours at 37° C., instead of the longer periods at lower temperature in more or less general use at the present time.

In conclusion the writers desire to acknowledge the courtesy of Mr. Julian Griggs, chief engineer of the Board of Public Service, in permitting the use of data embodied in this paper. They desire further to make acknowledgment of the suggestions and criticisms offered by Mr. George W. Fuller, who, as one of the consulting engineers to the city of Columbus on sewage disposal matters, has closely followed the work herein described from its conception.

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A COMPARATIVE REVIEW OF CURRENT METHODS FOR THE DETERMINATION OF ORGANIC MATTER IN SEWAGE.

George A. Johnson and A. Elliott Kimberly.
Columbus, Ohio.

During the past five years, largely through the efforts of the Committee on Standard Methods of the American Public Health Association,[1] methods for the determination of organic matter in sewage have been placed upon a more definite and uniform basis than was formerly the case. The Committee, while specifying certain methods as standard methods, are yet cognizant of the considerable variability in the practice in different places in this country, and the difficulties attending radical changes in existing methods in the case of the older and long-established laboratories.

With the view of bringing these different methods together in concise form, it is the purpose of the writers to compare briefly the current practices in use in the different laboratories in this country, in England, and in Germany. The discussion will be confined to the Kjeldahl process, the determination of nitrogen as free ammonia, oxygen consumed, and loss on ignition.

METHODS FOR THE DETERMINATION OF THE UNOXIDIZED NITROGEN.

The Kjeldahl process.—In the absence of a method whereby there may be obtained an accurate knowledge of the character and actual amount of the nitrogenous compounds which are normally present in sewage of domestic origin, the Kjeldahl process furnishes well-nigh indispensable information regarding the amount of nitrogen which occurs in sewage in complex atomic aggregations. This process, in use to some extent for a number of years, has finally become quite generally recognized as the most reliable means of determining the nitrogen of the organic matter, and as such has been recommended as a standard method for sewage work.

Standard American method.—The prescribed method for the determination of the total organic nitrogen in sewage embodies one or more of the salient features of the procedures used by Drown and Martin, Hazen,[2] and in England.[3] In the standard method, the nitrogen as free ammonia is first removed by distillation with steam, following which the sample is digested with sulphuric acid until colorless; a small crystal of potassium permanganate is added, the digestate made alkaline with an excess of sodium carbonate, and finally diluted to a volume of 500 c.c. A suitable volume of this solution is then distilled with steam, and the ammonia evolved determined in the usual manner. Catalytic agents are not used in this method, and no steps are taken for the preliminary reduction of nitrified nitrogen.

English methods.—The methods of analysis described by Fowler,[3] by Rideal,[4] and employed by McGowan[5] may be taken as representative of English practices in sewage analysis.

McGowan and his associates have used the Kjeldahl process to a considerable extent to determine both the total unoxidized and organic nitrogen in sewage. Their methods include both distillation and a direct nesslerization of the neutralized digestate. Their practice in regard to the Kjeldahl process is essentially different from that in common use in this country, in that a reduction of nitrate nitrogen is considered necessary. This is effected by a very slow reduction with zinc and sulphuric acid, requiring five days for its completion. Their reports distinctly state that when the reduction is omitted, or when a shorter period than five days is employed, loss of nitrogen is inevitable.

A direct process described by them involves a neutralization of the digestate, and the addition of sodium oxalate for the removal of the calcium, following which a suitable portion of the settled solution is diluted and nesslerized. This method is not recommended by McGowan for general use, owing to the liability to turbid tubes, and to the necessity of preliminary reduction of nitrates before the actual Kjeldahl digestion is attempted. Rideal,[4] however, describes a direct method of which he thinks well, without commenting upon the necessity for preliminary reduction of nitrate nitrogen. The tediousness of McGowan's method obviously detracts from its value for practical routine work.

German methods.—From the works of Farensteiner, Buttenburg, and Korn,[7] and the reports of the Royal Bureau of Sewage Disposal at Berlin,[8] we learn that the Kjeldahl process is there regarded as of great value in sewage work. The official method includes the destruction of all oxidized nitrogen by treatment with sodium sulphite, ferric chloride, and dilute sulphuric acid, after which follows the usual digestion, neutralization, and distillation. Owing to the high concentration of German sewages the final step is a distillation into standard acid rather than nesslerization. In some cases catalytic agents are used, such as platinic chloride and copper oxide,[9] or copper sulphate or oxide.[10] The German method of preliminary reduction is much more satisfactory than the tedious English method. Neither of these, however, is considered essential for American conditions.

Phelps' method.—In the work at the Sewage Experiment Station of the Massachusetts Institute of Technology, Phelps[6] has used a Kjeldahl method wherein copper sulphate is employed as a catalyzing agent and permanganate is added at the completion of the digestion. Phelps shows very clearly the necessity for carrying the digestion to a point where a colorless solution will result, and brings forward data as to the feasibility of using permanganate; wherefrom he concludes that permanganate, although not essential in all cases, must still be considered a necessary adjunct to the reagents used in the Kjeldahl process as employed by Palmer.[11] He concludes his paper with suggestions in favor of the feasibility of determining organic nitrogen by direct nesslerization of the ammonia thus formed.

Columbus method.[12]—Following lines suggested by the English workers, a method for the direct nesslerization of the neutralized digestate of the Kjeldahl process was developed at the Columbus Sewage Testing Station. By applying the principles of water softening, there were eliminated the calcium and magnesium, whose interference has long been the cause of the turbidity besetting former attempts at direct nesslerization. The details of the method are given in another paper, but, in brief, the neutralized digestate is first treated with an excess of alkali, sodium carbonate is then added to precipitate the calcium, and under these conditions complete softening of the digestate results, allowing the clear yellow color of the mercury ammonium iodide to develop in a normal manner upon the subsequent nesslerization of a suitable portion of the clarified digestate. Potassium permanganate is omitted on the ground that manganous salts might escape oxidation during the preparatory treatment of the digestate, whereby turbid tubes would result on nesslerization, as experiments to cover this point have clearly indicated.

THE NECESSITY FOR PRELIMINARY REDUCTION OF NITRATES IN THE KJELDAHL PROCESS.

Some published literature is available on the effect of nitrate nitrogen upon the results of the Kjeldahl process. Rideal[4] describes experiments wherein certain amounts of nitrate were added to sewages to the extent of 68 and 136 parts of nitrogen as nitrate per million, respectively. To these sewages the Kjeldahl process was applied, digesting with 2 c.c. and 4 c.c. sulphuric acid, respectively. The results of the experiment are shown in the following table, from which the conclusion was drawn that the large excess of sulphuric acid used in the Kjeldahl process prevents loss of nitrogen by secondary decomposition.

TABLE 1.

DR. RIDEAL'S EXPERIMENTS AS TO THE EFFECT OF NITRATES UPON THE RESULTS OF THE KJELDAHL PROCESS.

c.c. Sulphuric Acid Added	Total Nitrogen by Kjeldahl—Parts per Million		
	Parts per Million added Nitrate Nitrogen		
	0.0	68	136
2	425	350	375
4	410	375	390

NOTE.—Sample strongly urinous, contained 350 parts per million nitrogen as free ammonia.

Phelps, in a study of the Kjeldahl process, has published some convincing data with regard to the effect of nitrates. He added nitrate and nitrite nitrogen in varying amounts up to 40 parts nitrate and 20 parts nitrite per million, respectively, to samples of crude sewage, septic sewage, coarse-grain filter effluents, and sand filter effluents, and determined the unoxidized nitrogen in the several samples before and after treatment. The results showed conclusively that no practical interference need be looked for in the examination by the Kjeldahl process of samples containing as high as 60 parts nitrified nitrogen per million.

The writers have also made experiments upon this point, determining by the direct Kjeldahl process the unoxidized nitrogen in samples of a settled sewage to which nitrate nitrogen was added up to 30 parts per million. From the results given below it is evident that the effect of nitrate is practically negligible.

TABLE 2.

EFFECT OF NITRATES UPON THE RESULTS OF THE KJELDAHL PROCESS.
(Average of Duplicate Determinations.)

PARTS PER MILLION NITRATE NITROGEN ADDED	NITROGEN—PARTS PER MILLION		
	Total	As Free Ammonia	Organic
0............	15.4	6.4	9.0
10............	15.4	6.4	9.0
20............	15.0	6.4	8.6
30............	14.6	6.4	8.2

NOTE.—0.1 difference on standard is equivalent to 0.4 part per million for 50 c.c. sample.

RELATIVE SUPERIORITY OF THE KJELDAHL PROCESS OVER THE ALBUMINOID AMMONIA PROCESS.

The albuminoid ammonia process has been in general use for many years in this country and in England, but only in recent years has been supplanted in the larger laboratories by the Kjeldahl process. The former process has, as we all know, long been recognized as yielding uncertain results, which represent only a portion of the total organic nitrogen obtainable by the use of the Kjeldahl process. The variability of the ratio between the albuminoid ammonia and the Kjeldahl values was well illustrated by the comparative serial analysis made during the staling of a sample of fresh sewage, as reported by Fuller.[13] In this case the ratio varied from 20 per cent to 50 per cent, increasing as the septicity of the sewage increased.

The uncertainties of the albuminoid ammonia results have been shown by a number of observers.[14 15 16 17 18 19 20]

TABLE 3.

COMPARATIVE DATA ILLUSTRATIVE OF THE VARIABILITY OF THE RELATION WHICH ALBUMINOID NITROGEN RESULTS BEAR TO KJELDAHL NITROGEN.

CRUDE SEWAGE.

LOCALITY	OBSERVER	NITROGEN PARTS PER MILLION		PERCENTAGE WHICH ALBUMINOID NITROGEN IS OF NITROGEN BY THE KJELDAHL PROCESS
		As Albuminoid Ammonia	Kjeldahl	
Lawrence, Mass............	Clark	3.0	7.8	39
Hopedale, Mass............	"	2.2	6.0	37
Leicester, Mass............	"	3.3	7.2	46
Manchester, Eng............	Fowler	4.2	19.0	22
Manchester, Eng............	"	4.8	15.5	31
Belfast, Eng................	Letts	8.9	15.3	58
Saltley, Eng................	Watson	15.6	30.1	40
Rhea, Eng................	"	15.2	48.3	21
Hocklay, Eng............	"	16.7	59.7	28
Aston, Eng................	"	19.5	92.8	21
Columbus, Ohio*...........	Johnson and Kimberly	3.5	7.3	48

*Average of 22 analyses.

NOTES.—Massachusetts data compiled from Report Massachusetts State Board of Health, 1903. English data, from Manchester Reports and Royal Commission Reports, 1902, and Report of Birmingham, Rhea, and Tame Drainage Board, 1903

TABLE 4.

COMPARATIVE DATA ILLUSTRATIVE OF THE VARIABILITY OF THE RELATION WHICH ALBUMINOID NITROGEN RESULTS BEAR TO KJELDAHL NITROGEN.

SEPTIC SEWAGE.

LOCALITY	OBSERVER	NITROGEN. PARTS PER MILLION		PERCENTAGE WHICH ALBUMINOID NITROGEN IS OF NITROGEN BY THE KJELDAHL PROCESS
		As Albuminoid Ammonia	Kjeldahl	
Lawrence, Mass............	Clark	3.0	7.8	39
Hopedale, Mass............	"	2.2	6.0	37
Leicester, Mass............	"	3.3	7.2	46
Manchester, Eng............	Fowler	4.2	19.0	22
Manchester, Eng............	"	4.8	15.5	31
Columbus, Ohio............	Johnson and Kimberly	3.6	6.4	56
SEWAGE SLUDGE.				
Brockton, Mass............	Clark	56.0	181	31
Lawrence, Mass............	"	13,000.0	23,700	55
Lawrence, Mass............	"	390.0	860	45
Lawrence, Mass............	"	633.0	1.190	56
Lawrence, Mass............	"	731.0	1,644	44
Lawrence, Mass............	"	222.0	294	76
Lawrence, Mass............	"	530.0	943	56
EFFLUENTS OF COARSE-GRAIN SEWAGE FILTERS.				
Columbus, Ohio*...........	Johnson and Kimberly	1.1	2.1	52
Columbus, Ohio†...........		1.6	3.8	42

*Contact filter, average of 10 determinations.

†Sprinkling filter, average of 16 determinations.

From the Massachusetts and the English reports the tables on the preceding page have been prepared, which, while necessarily incomplete, may be said to illustrate the great variability of the ratio existing between the results by the two processes and the inherent cause of the decline of the albuminoid ammonia process.

METHODS FOR THE DETERMINATION OF NITROGEN AS FREE AMMONIA.

There are two methods for the determination of nitrogen in sewage in the form of ammonium salts, namely, by distillation with sodium carbonate or magnesia, or by direct nesslerization. The latter method is rapidly coming into general use. A digest of current thought and practice in this regard is as follows:

German method.—This method consists in a clarification of the sample with caustic soda. In certain cases zinc acetate is also added to remove hydrogen sulphide. Following this preparatory treatment, a suitable portion of the clear supernatant liquid is removed, diluted to 50 c.c., and nesslerized. Phelps[20] has modified the method by diluting the sample to double its original volume before adding the caustic soda, and by using copper sulphate and lead acetate as coagulants.

English method.—Rideal[4] and McGowan[5] describe a method for direct nesslerization. Rideal speaks highly of the process, while McGowan says it cannot be used in all cases, since turbid nesslerized tubes result under certain conditions, especially when hard effluents high in calcium are examined. There is an apparent lack of harmonious opinion in England regarding the feasibility of determining nitrogen as free ammonia by a direct process.

From our standpoint the cause of the complications referred to by McGowan are apparently due to the lack of sufficient carbonic acid ions to precipitate the incrusting calcium. In cases where a sewage high in sulphates is examined, in the absence of alkalinity, the technique should include the addition of sodium carbonates as well as caustic soda.

Columbus method.—An alternative method, applicable when the sewage contains high magnesium, consists in treating the sample with caustic soda in sufficient amount to precipitate the magnesium and to soften the sewage. A precipitate of magnesium hydrate and calcium carbonate results, whereby there is effected a complete clarification of the sewage. This modification is embodied in the Report of the Committee on Standard Methods.[1] For the typical hard sewage of Columbus, the presence of a lead salt was not required for the removal of sulphureted hydrogen. For sewages low in magnesium the method may be made applicable by the addition of a magnesium salt as a coagulant.

RELATIVE RESULTS BY THE DIRECT AND THE DISTILLATION METHODS.

The advantages attached to the direct methods of determining nitrogen in sewage in the form of ammonium salts refer to the fact that by distillation there is obtained a certain amount of ammonia, split off from partially reduced nitrogenous organic compounds on the border line between ammonium salts and crude organic matter. Further, as has been shown by Phelps,[20] distillation with sodium carbonate causes the development of ammonia from a number of nitrogenous substances. Under such circumstances a direct process should give somewhat lower results, representative of true ammonium compounds alone. As pointed out by Schmidtmann and Günther,[8] the direct processes yield results now higher, now lower, than the distillation process, but generally, according to the experience of the writers, averaging about 90 per cent of those obtained by distillation. In the following table are shown the results obtained by the writers upon this point.

TABLE 5.

COMPARISON OF RESULTS BY THE DIRECT AND DISTILLATION METHODS FOR THE DETERMINATION OF NITROGEN AS FREE AMMONIA.

CHARACTER OF SAMPLES	NUMBER OF SAMPLES AVERAGED	NITROGEN AS FREE AMMONIA PARTS PER MILLION		PERCENTAGE WHICH DIRECT RESULTS WERE OF DISTILLED RESULTS
		Direct	Distilled	
Crude sewage	32	13.1	15.1	87
Settled sewage	33	13.2	14.8	90
Septic sewage	27	12.8	14.0	90
Strained sewage	13	14.2	15.9	90
Effluent of coarse-grain filter	39	10.6	11.8	90
Effluent of sand filter	49	2.23	2.5	90

THE DETERMINATION OF OXYGEN CONSUMED.

No method has undergone greater modification than that for the determination of the oxygen consumed by, or absorbed from, potassium permanganate.

Standard method.—Following the practice of Palmer,[21] the Committee has recommended for use in newly established laboratories a 30 minute contact with acid permanganate in flasks immersed to the neck in a boiling water-bath. This period of contact and this technique were advised for the reason that a fairly complete oxidation is effected thereby, with a minimization of the personal errors incidental to the other modifications.

Lawrence method.—The practice at the Lawrence Experiment Station[2] is to boil for two minutes with an excess of permanganate, titrating back with oxalic acid.

Boston method.—This method[22] is similar to the Lawrence method except that the period of boiling is five minutes. In both these methods the permanganate is added to the hot solution.

German method.—In Germany[7] the period of boiling is uniformly 10 minutes, and the determination is usually made upon the *filtered* solution.

English methods.—In England the boiling methods are practiced but little, as considerable error is believed to be introduced through the decomposition of the permanganate itself at the temperature of boiling water. The English methods use the "thiosulphate-iodine" reaction for final titration, since oxalic acid is obviously unsuitable in the cold. The many modifications of the oxygen consumed test in current practice in England are as follows:

The "at once" test.[23]—In this method, carried out at room temperature, the excess of permanganate is removed within 30 seconds after its application by the addition of potassium iodine.

The "three-minute" test.—In this method[24] the permanganate is allowed a contact of three minutes at a temperature of 80° F.

Other tests.—Periods of 15 minutes,[25] two and a half hours,[4] and four hours, at 80° F.,[3] are in use in England. Of these, the 15 minute and four hour periods are the more generally practiced. These modifications are said to serve the purpose of differentiating the putrescible matter. As stated by Rideal,[4] at the Manchester Meeting of the Society of Chemical Industry in 1898, the several oxygen consumed tests were said to possess the following characteristics:

1. "The three minute test showed (nitrites, ferrous salts, sulphureted hydrogen and) putrefying matter decomposing permanganate at once with acid."

2. "The difference between three and 15 minutes showed matter readily putrefying and rapidly decomposing acid permanganate."

3. "The difference between 15 minutes and four hours gives matter capable of putrefying, though slow to decompose."

Rideal[4] obtains what he calls a final figure by subjecting the sample to the action of permanganate for two and a half hours at a temperature of 80° C.

COMPARISON OF RESULTS BY DIFFERENT METHODS.

These different methods for determining the oxygen consumed or absorbed from acid permanganate bear a fairly definite relation to one another, as is illustrated by the following table:

TABLE 6.

Comparison of Results by Different Methods.

Source of Method	Time of Contact (Minutes)	Temperature (°C.)	Relative Results to 5 Minute Boil—Authority		
			Columbus	Fuller	Kinnicutt
England	3	26.7	0.20	0.20	....
England	15	26.7	0.33	0.35	0.30
England	240	26.7	0.56	0.60	0.48
Palmer	30	96.0	3.14	....	....
"Absolute"	240	100.0	4.73	4.00	....
Lawrence	2	100.0	0.74	0.65	0.65
Boston	5	100.0	1.00	1.00	1.00
Germany	10	100.0	1.18	1.25	1.52

LOSS ON IGNITION.

The ignition of the residue remaining upon evaporation of a measured portion of sewage has been practiced for a good many years. The loss in weight sustained is roughly indicative of the total amount of organic matters present. As is well known, many other substances besides organic matter are volatilized during the ignition, so that the test does not strictly indicate the organic content of the sample. Notwithstanding the limitations of the method, the information afforded thereby is of considerable value in sewage work, as it is the only test whereby even an approximation of the total amount of organic matters present. Relative to questions of the disposal of sludge deposits, the data afforded have a particular field of usefulness.

Limitations of the process.—In brief terms, the loss in weight upon ignition includes all organic matters, a certain amount of carbonic acid and combined water split off from the carbonates of the alkaline earth metals, especially magnesium, and further, under certain conditions, a certain loss due to the deoxygenation of mineral salts in cases where the temperature of ignition has been too high. Chlorides are also a factor, when present in considerable amounts.

The factors of error have been corrected to a certain extent by the refinement of the technique of the method in its more recent development. At the same time, as is well known, the variable character of these complications cannot always be allowed for in an entirely satisfactory manner. Even under these conditions, the process is of considerable value, since it throws light upon those constituents regarding whose true nature, amount, and precise composition the present status of analytical methods yields but sparse information.

German method of determining loss on ignition.—During the tests made at Cologne, Germany, in 1904,[8] loss on ignition data was obtained by a technique involving repeated partial ignitions at a very low temperature (moving the dish back and forth over a low flame), preceded by the application to the residue of distilled water and subsequent evaporation. From four to six ignitions were usually made; following the final heating, the residue was treated with a few drops of ammonium carbonate solution to convert to carbonate any alkaline earth oxides. With this technique, a very complete combustion of the organic matter was effected, with a minimum loss of volatile mineral matter. This method appears to be a distinct improvement over the technique employed in this country, and, as brought out in another paper,[26] the experience of the writers indicates that a re-application of distilled water to ignitions made in the radiator following a second ignition might with advantage be included in the standard procedure, as the combustion of the organic matter would thereby be more complete and the results of the process more reliable.

Greater relative value of volatile suspended matter data.—Loss on ignition is of course not a test of differentiation. Although the data obtained by ignition of the residue from the evaporation of a measured amount of sewage are to be regarded only as approximations of the constituents they are intended to represent, it is possible, by the use of an entirely different process, to obtain precise information regarding that portion of the suspended matters in sewage which is of a volatile character. To effect this, the suspended matters are separated from the dissolved matters by filtration through asbestos in a Gooch crucible.[26] In the absence of the complicating mineral matters, the volatile suspended matters may be determined by an ignition at a temperature sufficiently high to effect a complete destruction of all organic matters. In problems relating to the preparatory treatment of sewage, these data are of great value, since they indicate to a certain degree the relative composition of the deposits of sludge which are incidental to these processes.

CONCLUSIONS.

Casting up the evidence, it seems apparent that more satisfactory practical results may be obtained by the Kjeldahl process than by the use of the albuminoid ammonia process, the results of which are such uncertain percentages of the total organic nitrogen present in sewage and in effluents.

The direct process for the estimation of the ammonia formed in the Kjeldahl method seems practical and reliable above 0.5 part per million organic nitrogen. Below this amount the distillation method of Palmer is available.

The direct determination of nitrogen as free ammonia is safe and accurate; either modification of the process may be used according to local hardness conditions.

Although governed largely by local conditions, the oxygen consumed test, with its many modifications, is to be considered of great value in the examination of crude and purified sewages.

Regarding the loss on ignition, the indications are that the total and volatile suspended matters, as determined directly by the Gooch process, are more reliable and more satisfactory data for sewage problems than either the total volatile matters in the sewage or effluent or the indirectly determined total and volatile suspended matters. The practical advantages of the direct Gooch process render the method worthy of serious consideration, especially in problems wherein suspended matter is an important factor.

None of the current methods for the determination of organic matter distinguish between stable and unstable organic matter. Only by resorting to one of the various methods for the determination of putrescibility can information be obtained regarding the putrescible and non-putrescible matter in partially purified sewage, and even under these conditions there is indicated only the relative predominance of putrescible and non-putrescible substances. It seems to be in this direction that the need is most urgent for methods applicable to the determination of the organic matter in crude and purified sewages.

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A METHOD FOR THE DIRECT DETERMINATION OF ORGANIC NITROGEN BY THE KJELDAHL PROCESS.

A. ELLIOTT KIMBERLY AND M. G. ROBERTS,
Columbus, Ohio.

ONE of the most conspicuous recent advances made in sewage analysis in this country may be said to be the development of a direct process for the determination of the nitrogen as ammonia.[1] The greater simplicity of the technique, the greater economy of time effected by the elimination of distillation, and the relatively greater accuracy of the process are salient advantages of the direct method which surely cannot fail to be of moment in the routine work of sewage laboratories.

To be able to apply a direct process to the determination of the ammonia formed in the determination of total organic nitrogen by the Kjeldahl process seemed the next step toward greater simplicity and rapidity of execution. During a considerable portion of the work at Columbus a direct process was in daily use for the determination of the nitrogen as ammonia, and a more intimate knowledge was gained of local conditions as to the disturbing factor of turbidity, to which, as we all know, has been chiefly due the indifferent success attending earlier attempts at the direct nesslerization of the acid digestate of the Kjeldahl process. Under such circumstances it was thought that a direct method for determinating nitrogen by the Kjeldahl process might be successfully developed.

Working along lines by which a direct process for the determination of the nitrogen as free ammonia was successful under such unusually hard water conditions as prevailed at Columbus, a method was finally obtained by which the disturbing turbidity was completely eliminated. It is our purpose to discuss the several factors which have to do with the turbidity occurring in nesslerized tubes, and further to describe a direct method for determination of nitrogen by the Kjeldahl process, found applicable under Columbus conditions, together with certain suggestions whereby it appears that the direct method may be made of quite extended applicability.

METHODS PREVIOUSLY PROPOSED.

A review of the literature upon the question of the direct nesslerization of the acid digestate of the Kjeldahl process indicates that the matter has received but little attention. So far as we are aware, the method described by Rideal,[2] a similar one used to some extent by McGowan,[3] and the modifications suggested by Phelps,[1] are the only instances on record in which the feasibility has been suggested of the elimination of distillation in Kjeldahl nitrogen determinations. Rideal states that the direct determination of the organic nitrogen has been practiced to some extent in England. The method as given by him is as follows:

> "*Kjeldahl.*—10 c.c. of a sewage, or say, 100 c.c. of an effluent plus 4 c.c. of pure sulphuric acid, are heated in a pear-shaped hard glass flask in a slanting position until the liquid becomes colorless. When about 2 or 3 c.c. remain, the flask is cooled and is washed out with small quantities of ammonia-free water into a 100 c.c. measure, until the volume of the liquid reaches about 40 c.c. An excess, i. e., about 25 c.c., of soda solution (25 per cent) free from ammonia is now added, when a flocculent precipitate is thrown down. After cooling, the liquid is made up to 100 c.c., transferred to a clean and dry stoppered bottle, and shaken at intervals until the flocculi—which at first float entangled with air bubbles—subside. A suitable fraction of the clear liquid is then pipetted into a Nessler glass, diluted to 50 c.c., and nesslerized. *This gives the total unoxidized nitrogen in terms of ammonia.*"

This method was used by McGowan,[3] modified only by the occasional addition of potassium oxalate solution to the alkaline digestate to precipitate the calcium, and by the proviso that the solution should be "rendered *just* alkaline with purified potash." The method is criticised by McGowan on the ground that turbidity is oftentimes noted, even when the calcium has been removed by the addition of oxalate solution, and that considerable entrainment of ammonia results from the precipitate formed upon the neutralization of the digestate. His chief objection to the process seems to be that where considerable amounts of nitrified nitrogen are present, all of the unoxidized nitrogen is not recovered, since, during the last stages of the digestion, a portion of this nitrogen is oxidized by the nitric acid present and is probably lost in the form of lower oxides of nitrogen. For this reason the English practice in regard to the Kjeldahl method for effluents includes a reduction of all nitrified nitrogen by the use of zinc and sulphuric acid. This portion of the procedure is stated by English workers to require five days for its completion; for otherwise, as their experience indicates, an appreciable loss of nitrogen will ensue. The discussion is concluded with the statement that although the direct method—with no reduction of nitrate—has been found at times to yield very satisfactory results in the analysis of very pure effluents, and frequently in others containing as high as 5.0 parts per million nitrogen as nitrate, yet, for general work upon sewage and sewage effluents, experience seems to be against the method, since no preliminary reduction of nitrate is included in the technique.

In this country, Phelps has outlined a method which he used with somewhat indifferent success, owing, as he states, to certain factors associated with the turbidity of the nesslerized tubes, which as yet are not clearly understood. The method employed by him consisted in making up the digestate to 250 c.c., removing 2 c.c., neutralizing, making up to 50 c.c., and directly nesslerizing.

In the nitrogen work at the Columbus Sewage Testing Station a study was made of the feasibility of adopting a direct method for the determination of the ammonia formed in the Kjeldahl process, along the lines suggested by the studies of previous workers to which we have just referred. With some modifications, the methods described by Rideal and McGowan have been found to yield satisfactory results when applied to the sewage and effluents under study at Columbus. Those changes were the results of an extended inquiry into the causes of the disturbing turbidity which, as we all know, has been the chief drawback to the applicability of the direct process. The results of these studies have been incorporated in the technique of the method as given below, and it is felt that the method, when modified along lines to be suggested in order to meet different conditions, gives promise of a fairly wide field of applicability in the analysis of sewage and sewage effluents.

FACTORS ASSOCIATED WITH THE TURBIDITY PRODUCED BY DIRECT NESSLERIZATION.

The cause of the disturbing turbidity which appears upon the addition of Nessler's reagent to solutions containing other salts as well as ammonium salts, has been chiefly attributed to the presence therein of calcium salts. Considering for the moment such conditions as would obtain in direct nesslerization, without treatment with caustic soda, of an aliquot portion of a hard sewage containing the bicarbonates of calcium and of magnesium, respectively, and, in addition, both these metals as incrustants, it follows from water-softening experience that the addition of the caustic Nessler's reagent will entail a precipitation of a portion of the calcium as carbonate, and the whole of the magnesium as hydrate, whereby cloudiness would result. Such seems to be one of the explanations of the failure of attempts to obtain clear tubes when small amounts of a hard sewage, or sewage containing but moderate amounts of the alkaline earth metals, diluted to the usual 50 c.c., have been directly nesslerized, or when, as in Phelps's method, a small portion of the acid digestate is neutralized and nesslerized without preparatory treatment for the removal of the magnesium, and in certain cases of calcium as well.

The method as advised by the Committee on Standard Methods for the determination of the nitrogen as free ammonia by direct[4] nesslerization, appears to admit of adoption under a wide range of conditions with respect to the mineral constituents of the sewage, for the reason that the preparatory treatment of the sample with caustic alkali effects a complete softening of a hard sewage, removing to a sufficient degree the carbonate of calcium, all of the magnesium, and a variable proportion of calcium sulphate, depending upon the relative amount of half-bound carbonic acid present. The development of a successful method for the direct nesslerization of ammonia has clearly resulted from the use of a preparatory treatment which softens the sewage materially, thereby removing before nesslerization those substances which, by precipitating in the nesslerized tubes, caused the turbidity so often observed.

Coming now to the premises of the paper, the thought arises as to wherein the conditions differ from those obtaining in the successful direct determination in hard sewages of the nitrogen as free ammonia. As is well known, under certain conditions the metals of the alkaline earth group, when present in a nesslerized tube, obscure by precipitation the clear yellow of the mercurio-ammonium compound, and thus the question of the turbidity of the neutralized and nesslerized acid digestate would appear to revert to the interference of calcium and magnesium salts, and, further, possibly to the presence of sodium sulphate from the neutralization of the sulphuric acid.

A little thought will suggest that, irrespective of the nature of the acid radicals to which the calcium and the magnesium are united in the sewage, they are in combination, after digestion, with sulphuric acid as sulphates. The neutralization of the acid digestate with a sufficient excess of alkali removes the magnesium as in the direct ammonia procedure, so that, so far as these metals are concerned, the question of turbidity would appear to refer to the presence, of calcium as sulphate, which is not removed upon the neutralization of the acid digestate with carbonate-free caustic soda. We have found by experiment that calcium as sulphate may be present in a nesslerized tube without causing precipitation, to the extent of 50 parts per million of calcium (Ca). Above this amount, up to the limit of the solubility at ordinary temperatures of calcium as sulphate of 100 parts per million of calcium (Ca), turbidity will be noted. Under ordinary conditions, calcium in this form is probably not a factor; but to avoid possible complications, under conditions to be referred to later, it would seem advisable to add to the digestate a certain amount of carbonate of soda, for the removal of the calcium, imitating conditions obtaining in most cases in the direct nesslerization of the nitrogen as ammonia. To study the effect of the presence of sodium salts, a blank digestate was neutralized and carried through the usual procedure, as given below, and in no case did any turbidity appear to be traceable to this source.

Taking into account each of the factors just discussed, efforts to obtain clear tubes continued to be indifferently successful, until finally it developed that the character of the caustic soda used for neutralization was related to the intensity of the turbidity of the nesslerized tubes. The so-called C. P. stick caustic soda is far from pure, as is well known, and it was found that the usual treatment for the removal of ammonia was not sufficient to prevent a precipitation in nesslerized tubes containing free alkali. To remove ferrous iron, organic matter, or any chance reducing agent to any of which the turbidity would appear to be due, caustic soda was prepared, by oxidation with either sodium peroxide or permanganate, as described below. With caustic solutions prepared in this manner, fairly satisfactory results were obtained.

Up to this time the method described by Palmer[5] had been used, wherein permanganate is added to the digestion when it is judged to be complete. In reviewing possible causes of the disturbing turbidity, the thought suggested itself that the presence of manganous sulphate, even in small amounts, would tend to produce turbidity, because of the ease with which the salt is oxidized and precipitated in alkaline solutions. Experiments with solutions containing manganous sulphate (formed when permanganate is used) showed conclusively, as was expected, that turbid tubes would result upon nesslerization. As the use of permanganate is of uncertain value as an aid in the oxidation by sulphuric acid of the substances found in ordinary sewage, following the practice of Rideal,[2] the addition of permanganate at the end of the digestion was omitted from the procedure as finally adopted, on the ground that a certain amount of manganous sulphate might escape oxidation during the preparation of the digestate for nesslerization, whereby turbid tubes would result.

By the use of a caustic soda solution free from oxidizable organic matter, clear tubes were finally obtained, provided that a sufficient excess of caustic was added to precipitate completely the magnesium, and provided that sodium carbonate was added to remove the calcium.

Considerable literature is available upon the use of Nessler's reagent, and from the work of Nessler,[6] Miller,[7] Chapman,[8] Frankland and Armstrong,[9] Trommsdorff,[10] and Fleck,[11] it appears that both free alkali and the presence of magnesium, or the formation in the tubes of any other precipitate whatsoever, affects the color produced by the reagent. The alkali tends to give abnormally high readings, and the co-precipitation of any other substance, together with the mercury ammonium iodide, completely coagulates the colloidal color produced by Nessler's reagent in pure solutions of ammonium salts. In connection with the question of the direct nesslerization under hard water conditions at least, it is a significant fact that the use of a magnesium salt forms the basis of Fleck's titration method[11] for the determination of ammonia by Nessler's reagent, wherein the mercury ammonium iodide, completely precipitated by magnesium, is dissolved in sodium thiosulphate and titrated with an ammoniacal solution of silver sulphide.

It is evident that an excess of alkali will remove the magnesium. To determine the limit to which the excess could be carried with safety, different amounts of differently prepared caustic soda solutions, ranging by 10ths to one gram, respectively, were added to Nessler tubes containing 3 c.c. of ammonia standard. The tubes were filled to the mark with ammonia-free water, and were then nesslerized. The colors were read after 15 minutes. It appears from the results in the table below that 0.2 gram of pure caustic (NaOH) may be present in a nesslerized tube without producing turbidity or causing the color to be abnormal. Beyond this amount, turbidity, and finally complete precipitation, will result, as will also be the case when impure caustic is used without an oxidizing treatment.

Above 0.5 gram a heavy precipitate was observed in all cases.

TABLE 1.

EFFECT OF THE PRESENCE OF CAUSTIC SODA UPON THE COLOR PRODUCED BY NESSLER REAGENT.

CAUSTIC SODA (NaOH) (Gram)	TREATMENT RECEIVED BY CAUSTIC*							
	NONE				SODIUM PEROXIDE		PERMANGANATE	
	FUSED STICK		BY ALCOHOL					
	Reading	Turbidity	Reading	Turbidity	Reading	Turbidity	Reading	Turbidity
0.0	3.0	0	3.0	0	3.0	0	3.0	0
0.1	...	str.	3.0	0	3.0	0	3.0	0
0.2	...	“	3.0	0	3.0	0	3.0	0
0.3	...	“	3.1	0	3.1	0	3.2	0
0.4	...	“	3.5	sl.	3.3	v. sl.	3.5	v. sl.
0.5	...	“	3.8	str.	3.5	str.	3.6	str.

*Fused white sticks.

By including in the final details of the method an excess of alkali corresponding to 2 c.c. of 25 per cent caustic soda, or 5.0 gram of the alkali giving in the nesslerized tubes only one-tenth of this amount and adding 0.2 gram of sodium carbonate to the alkali digestate, clear tubes were finally obtained. The method employed takes into account the following factors, which our experience indicates as the controlling causes of turbid tubes in direct nesslerization work:

a) Insufficient preparatory treatment of the sample, whereby there results a precipitation of calcium carbonate or of magnesium hydrate, and, in general, the formation by the alkali of the Nessler's reagent of any other precipitate whatsoever save the normal colloidal mercury ammonium iodide.

b) The use of caustic soda containing organic matter.

c) The presence of a manganous salt from the use of permanganate in the digestion.

d) The presence in the nesslerized tubes of calcium as sulphate in hard sewages above 50 parts per million, and, further, the neglect to mix thoroughly the solution before nesslerizing, even in cases where the calcium in a tube containing a homogeneous solution would be below the limiting value of 50 parts per million.

DETAILS OF THE COLUMBUS METHOD FOR THE DIRECT DETERMINATION OF NITROGEN BY THE KJELDAHL PROCESS.

Although subject to considerable modifications from time to time, to correspond to developments in the study of the turbidity question, the Columbus method for the direct determination of nitrogen by the Kjeldahl process finally assumed the following definite shape:

Reagents.—Aside from the preparation of the caustic, the use of a sodium carbonate solution, and the omission of permanganate, the reagents do not differ from those used in the standard Kjeldahl process.[5] Two brands of caustic soda were used, one the ordinary fused white sticks, the other caustic soda from alcohol. Both require a certain amount of oxidation to remove all organic matter. This has been effected either by the addition of about 2 per cent of sodium peroxide to the caustic solution prepared from caustic by alcohol, or, in case the ordinary fused white stick caustic, which is somewhat cheaper than that from alcohol, is used, by the use of potassium permanganate as follows:

Caustic soda solution: Dissolve in a liter of redistilled water 250 grams of fused white stick caustic soda. Heat to boiling and add a strong solution of potassium permanganate, drop by drop, until a pale-green color persists; continue to boil to expel ammonia and to cause a precipitation of the manganic oxide.

If the conditions are right, the caustic solution will assume a coffee-brown color, due to the suspended manganic oxide. In cases where the caustic is desired for immediate use, the solution, when cooled sufficiently, is filtered through asbestos by the aid of a filter pump; the resulting filtrate should be perfectly colorless, and free from suspended matter. Where time permits, the turbid solution may be allowed to stand until the precipitate shall have subsided.

Carbonate of soda solution: Dissolve 100 grams of C. P. anhydrous salt in one liter of ammonia-free distilled water.

Method.—Measure out such an amount of the sample as will contain between 0.00050 and 0.00100 gram of nitrogen; that is, an amount which will be equivalent to from 50 to 100 c.c. of the nitrogen ammonia standard; and digest with 5 c.c. of sulphuric acid, until the liquid is colorless or, in the presence of iron, shows a pale-yellow color which becomes white when the solution has cooled. Rinse the acid digestate into a 50 c.c. flask, allow to cool to room temperature, fill to the mark, and mix thoroughly by inverting the flask at least four times. Place 25 c.c. of this solution in a 100 c.c. flask, preferably glass-stoppered, and add an amount of caustic soda solution not quite sufficient to neutralize the acid completely. Immerse the flask in ice-water to effect a rapid cooling. When cool, add more caustic soda solution from a pipette, drop by drop, until a flocculent precipitate is plainly visible. Add then 2 c.c. of the sodium carbonate solution. Make up to the 100 c.c. mark, stopper the flask, and mix thoroughly. Pour the solution into a clean four-ounce white glass bottle, preferably tall, and allow to stand at least six hours, shaking slightly at intervals in case the flocculi do not tend to settle readily. Pipette 10 c.c. of the completely clear, colorless supernatant solution into a 50 c.c. Nessler tube, make up to the mark with ammonia-free water, and mix thoroughly by inverting the tube. Nesslerize as usual and read after 15 minutes. The nesslerized tubes should show no turbidity. As in all Kjeldahl methods, a reagent blank must be subtracted from each determination. The results are computed as follows:

$$\frac{(N \times 20) - \text{Blank}) \times 1{,}000{,}000 \times .00001}{S}$$

Where N=c.c. standard reading
S=c.c. sample digested.

The results are in terms of the *total unoxidized nitrogen* in the sample. The *organic nitrogen* is determined by subtracting from these results the amount of ammoniacal nitrogen determined by a direct process.[12]

DISCUSSION OF CERTAIN IMPORTANT FEATURES OF THE METHOD.

There are several points relative to the method, which our experience has shown to be very essential to the attainment of satisfactory results.

Turbidity.—To avoid cloudy tubes a period of subsidence of at least six hours is necessary both to insure a clear supernatant solution and also to effect a complete precipitation of the slowly forming calcium carbonate. Further, sufficient carbonate of soda must be present to precipitate the calcium, and a sufficient excess of purified caustic soda solution is necessary for the complete removal of magnesium. An excess of 0.05 gram caustic soda (NaOH) was found to be sufficient. Since, as pointed out above, 0.2 gram of free caustic alkali does not appear to interfere with Nessler's reagent, 0.05 gram of alkali, the amount in each tube, may be safely used, thus insuring a complete precipitation of magnesium.

Neutralization.—The neutralization has been completed in two stages, cooling the solution before alkalinity obtains to guard against any possible loss of ammonia. Ice-water has been found convenient as a cooling agent.

Apparatus and technique.—In the technique of the method, ordinary graduated volumetric flasks have been used for making the necessary measurements, and for mixing the several solutions. For general practice it would certainly be advantageous to use glass-stoppered 50 and 100 c.c. flasks, respectively, in order that mixing may be carried on without contamination and without injury to the hands.

The containers in which the neutralized digestates are allowed to settle should preferably be tall, in order to facilitate the subsequent pipetting of the supernatant solution. During subsidence, the bottles should be protected from dust and carbonic acid, and to this end it is suggested that glass-stoppered bottles of about 120 c.c. capacity, contained in suitable racks, could be advantageously used to protect the determination prior to the completion of the process.

RELATIVE RESULTS FROM THE DIRECT AND DISTILLATION PROCESS, RESPECTIVELY.

In the work at Columbus, before substituting the direct method for the distillation process in current use, a sufficient number of comparative analyses were made to insure that the results should be strictly comparable to those of the older method. At the same time, it was thought that the simpler technique of the direct method and the elimination of all factors of error associated with the distillation might be the means of giving somewhat higher results. Representative results are shown in the table below, from which it appears that the results by the direct process are substantially the same as those obtained by the distillation of the neutralized digestates. The differences observed in the results by the two methods are so slight as to be outweighed, from a practical standpoint, by the very great saving of time effected by the use of the direct process.

ENTRAINMENT OF AMMONIA.

One of the objections to the direct process raised by McGowan[3] refers to the possible loss of ammonia through absorption by the precipitate formed when the acid solution of the digestate is neutralized, or when, as in the direct determination of the nitrogen,[4] as free ammonia a more or less heavy precipitate forms upon the addition of alkali. McGowan made experiments to illustrate this by nesslerizing a

TABLE 2.

REPRESENTATIVE ORGANIC NITROGEN RESULTS BY KJELDAHL—COMPARISON OF THE DIRECT AND DISTILLED METHODS.

Parts per Million—Nitrogen.

SOURCE OF SAMPLE	ORGANIC	
	Direct	Distilled
Crude sewage	10.1	9.7
	4.5	4.5
	9.0	8.6
	10.3	9.9
Settled sewage	4.9	4.7
	10.7	10.3
	5.1	5.1
	13.9	14.0
Septic sewage	5.4	5.4
	4.7	5.2
	5.4	5.1
	7.0	7.4
Coarse-grain filter effluent	4.7	5.2
	3.9	3.9
	2.2	2.2
	1.8	1.8
Ditto settled	3.5	3.7
	1.8	1.9
	2.1	2.2
	3.6	3.0
Sand filter effluents	0.67	0.66
	0.37	0.44
	0.53	0.50
	0.65	0.54

portion of the clear liquid in the direct Kjeldahl process, then shaking up the precipitate in the remaining liquid, subjecting the turbid solution to distillation, and determining the amount of ammonia in an aliquot portion of the distillate. He shows that an appreciable entrainment of ammonia results, but adds that a stricter comparison would have been effected had both the clear and the turbid liquid been subjected to distillation. Phelps,[1] in a recent paper upon the determination of ammonia in sewage, alludes to the entrainment factor, which he substantially eliminates by diluting the sample in which it is desired to determine the nitrogen as free ammonia before the addition of alkali.

Entrainment experiments.—To study this point under Columbus conditions, a portion of the clear supernatant solutions from the nitrogen as free ammonia and from the total nitrogen determinations, respectively, were nesslerized as usual, following which 40 c.c. of the alkaline solutions, containing probably all of the precipitate, were respectively dissolved in small amounts of sulphuric acid. The solutions were then diluted to 100 c.c., and the calcium and magnesium salts again precipitated by the addition of caustic soda. Aliquot portions of the clear supernatant solutions, corresponding in amounts of ammonia to those in the original determinations, were then diluted to 50 c.c., and the ammonia determined as usual. If the entrainment factor were one of appreciable moment, it was thought that the ammonia reading in the tubes from the second precipitation would be correspondingly higher than those obtained in the original determination, since the second digestion contained practically all the precipitate formed in the initial precipitation. Since practically the same readings were obtained in both cases and in both of the direct methods, the indications are that the entrainment factor is not of sufficient moment, from a practical standpoint, to render the results of direct processes unreliable. Experimental results upon the absorption factor are shown in the following table:

TABLE 3.

EFFECT OF ENTRAINMENT UPON RESULTS OF DIRECT NESSLERIZATION.

Nitrogen as Free Ammonia—Direct Kjeldahl.

NUMBER	STANDARD READING			
	A	B	A	B
1	4.0	3.9	2.4	2.4
2	3.5	3.5	2.9	2.9
3	3.5	3.5	2.2	2.2
4	3.1	3.1	2.9	2.7

NOTE.—A=Supernatant from first precipitation; B=ditto from second precipitation.

DISCUSSION OF THE GENERAL APPLICABILITY OF THE DIRECT PROCESS IN SEWAGE WORK.

In considering the method for the direct determination of the total unoxidized nitrogen by the Kjeldahl process, which has been found applicable for Columbus conditions, where unusually large amounts of calcium and magnesium are encountered, with respect to its applicability for sewages common to New England and to other places where soft water conditions are as a rule more in evidence, Columbus experience leads us to believe that the method can be successfully used. Since a digestate must be freed from the alkaline earth metals before a direct treatment with Nessler's reagent is attempted, it would seem, in the absence of the complicating salts of hard sewages, that no difficulty would be experienced in obtaining clear tubes.

In this connection it may be stated that the direct process has been applied in the Columbus work to the determination of total nitrogen in bacterial culture media work, in connection with studies of denitrification such as have recently been brought forward at the Lawrence Experiment Station.[13] Excellent results were obtained by the direct nesslerization of aliquot portions of neutralized digestates, in which the Gunning modification[14] of the Kjeldahl process was employed.

For soft-water sewages the method appears to be applicable. Even for harder sewages than a maximum of about 600 parts per million as calcium carbonate, encountered under Columbus conditions, it is thought that the calcium in solution as sulphate would be reduced below 50 parts per million in the nesslerized tubes by the addition of sodium carbonate, and in view of the fact that calcium as sulphate is not soluble over 100 parts per million[15] in pure water and probably much less so in solutions of sodium sulphate. In the case of a very hard sewage, or where a moderately hard sewage low in nitrogen is examined, as we have often noted, considerable calcium sulphate separates out when the digestate is diluted to a volume of 50 c.c. In all cases, therefore, the added sodium carbonate should be sufficient to remove all of the interfering calcium, even when present to the limit of its solubility.

APPLICATION TO WATER ANALYSIS.

Outside of the field of sewage analysis is the question of the feasibility of using a direct process in the sanitary analysis of water, in those cases where the total organic nitrogen is determined by the Kjeldahl method. No literature upon this matter has come to our attention.

By modifying the technique to include the neutralization of the entire digestate, with the addition of the usual excess of caustic soda, by adding a suitable amount of sodium carbonate, and by nesslerizing up to 50 c.c. of the alkaline digestate, it appears highly probable that a direct process might be used in water work. We would be inclined to place the limit of total nitrogen obtainable with the direct process at 0.1 part per million.

For such small amounts of total unoxidized nitrogen, complete softening is absolutely essential to the success of direct nesslerization. Moreover, since the direct process for the determination of the nitrogen as free ammonia cannot be applied to the determination of such small amounts as usually accompany low total unoxidized nitrogen values, the advantages of a direct process are not so apparent, especially since distillation processes give small amounts of ammonia with such relatively greater accuracy than can be obtained by a direct process, in which errors of multiplication are always an accompanying feature. It is felt, however, that the direct process may have a certain field of usefulness for fairly polluted waters, and it is hoped that the suggestions made herein may be of some value to other workers, to whom the opportunity may be given for studying the further development of the direct process along lines which we have not been able to pursue under the hardness conditions obtaining at Columbus.

In conclusion, we desire to express our thanks to Mr. Julian Griggs, chief engineer of the Board of Public Service, Columbus, Ohio, by whose permission we have been enabled to present the results of these analytical studies before the publication of the report of the Testing Station. We wish also to express our appreciation of the valued criticisms of Mr. George W. Fuller, consulting engineer, and the valuable suggestions of Mr. George A. Johnson, engineer in charge of the Testing Station.

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THE PRACTICAL ADVANTAGES OF THE GOOCH CRUCIBLE IN THE DETERMINATION OF THE TOTAL AND VOLATILE SUSPENDED MATTER IN SEWAGE.

A. Elliott Kimberly and Harry B. Hommon,
Columbus, Ohio.

The determination of the total and volatile suspended matter in sewage and sewage effluents has recently become of considerable importance, owing to the rapidly increasing number of sewage disposal problems in which conditions necessitate the adoption of filters of coarse-grain material, generally preceded by settling or septic tanks. In determining the efficiency of these latter-day sewage works, and in special investigations incidental to the design of plants of this character, it is highly important that accurate data be obtained upon the amount and character of the suspended matter in the raw sewage. For it is this point which, in a very large measure, controls decisions regarding the character of the preparatory treatment which is best fitted to a given set of conditions, and to which are closely related, also, decisions regarding the character of the finishing process.

In the course of the investigations at the Testing Station, Columbus, Ohio, the question of the removal of the suspended matter in crude sewage under different velocities was made the subject of special study, as will be reported elsewhere, necessitating a large number of determinations of both total and volatile suspended matter. Owing to the pressure of other lines of routine analytical work, and the large amount of expensive apparatus necessary in connection with the usual indirect platinum evaporation method for work of this character, the direct Gooch crucible method, used to advantage by McGowan[1] in sewage work, was substituted in a material measure for the more tedious indirect platinum method. Our experience in this connection indicates that this direct method possesses advantages sufficiently distinct to warrant its serious consideration by other workers interested in the determination of total and volatile suspended matter in sewage.

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[1] *Fourth Report Royal Com. on Sewage Disp.*, 4. 30, Pt. 5, p. 47.

So far as we are able to learn, the application of the Gooch crucible to the determination of the suspended matter in water was first suggested by Thomas and Hall[1] of Philadelphia; in sewage analysis, so far as we are aware, it was developed by McGowan, as described in detail in the *Chemical Report to the Royal Commission on Sewage Disposal*, 1905. The author states, as a result of his experience, that the Gooch crucible may be relied upon to give very accurate data upon suspended matter in sewages, with a minimum expenditure of time. We have carefully studied, under Columbus conditions, the reliability and the general practical advantages of this method for the determination of suspended matter in sewage and sewage effluents, and our results indicate the correctness of the assertions of the English workers.

This paper will be devoted chiefly to a detailed account of our experience with the Gooch crucible in sewage work, referring to the determinations of the volatile, as well as to the total suspended, matter, together with a discussion of the relative merits, from a practical standpoint, of the Gooch crucible and the indirect platinum methods.

DETAILS OF THE COLUMBUS METHOD FOR THE DETERMINATION OF THE TOTAL AND VOLATILE SUSPENDED MATTER BY THE GOOCH CRUCIBLE.

Preparation of asbestos.—Asbestos adapted for use in the Gooch crucible may be readily prepared from the granular commercial product by digestion on a water bath in strong hydrochloric acid for several hours.[2] By successive decantations with distilled water, the acid is completely removed, leaving the asbestos practically free from iron. High-grade, long fiber asbestos has not been found to yield as good results as the commercial asbestos purified as above described.

Preparation of mat.—Prepare a dilute cream of the washed asbestos, which must be free from coarse particles, attach the crucible* to the filter flask in the usual manner, start the suction, and form a mat about $\frac{1}{16}$ inch thick upon the bottom of the crucible. After the asbestos has drained completely, apply to the crucible a small quantity of distilled water. If the mat is of the correct thickness, the distilled water will pass through the filter at the rate of about 50 drops per minute. Place the crucible in an oven at 110°–120° C. for 15 minutes; remove and ignite in a radiator for five minutes; cool in a desiccator and weigh. Before each weighing it is expedient to cleanse thoroughly with a soft cloth the outside surfaces of the crucibles. In the absence of a platinum radiator, we have found that a four-inch nickel dish, heated to a dull red heat, admirably serves the purpose of this method. During the ignition it has been the practice to allow the crucible to rest directly upon the bottom of the radiator

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[1] *Jour. Am. Chem. Soc.*, 1902, 24, p. 538.

[2] *Fourth Report Royal Com. on Sewage Disp.*, 4, Pt. 5, p. 47.

*Royal Berlin solid porcelain crucibles were used for this work.

Filtration.—Use 50 c.c., 100 c.c., or more of the sample, decanting into the crucible as great an amount as possible of the supernatant water before the main portion of the suspended matter is applied thereto; in this way the filtration will be the more rapidly accomplished. Allow the liquid to disappear completely before adding subsequent portions of the sample, also to facilitate filtration. When the filtration is completed, rinse out the flask with about 15 c.c. of distilled water. To guard against imperfect filtration, it is advisable to apply the suction gradually. In case the filtrates are cloudy, they must be refiltered until clear. With a properly prepared mat, our experience indicates that imperfect filtration is of rare occurrence.

Drying and igniting.—The crucible is dried at 110°–120° C. for one hour, cooled in a desiccator, and weighed, the increase in weight representing the *total suspended matter* in the sample. To obtain the *volatile suspended matter*, the weighed crucible is ignited in the radiator at a low red heat, for 10 minutes or to constant weight. The completeness of the ignition may be judged by the appearance of the residue. The suspended matter in sewage work is usually black in color, owing to the presence of sulphide of iron. On ignition, as is evident, this is oxidized to ferric oxide, which usually predominates sufficiently to impart to the thoroughly ignited residue, when cool, a reddish-brown color. Our experience indicates that an ignition of 10 minutes is sufficient to effect a complete oxidation of the organic matter present.

Removal of mat.—To prepare the crucibles for further use, remove the mat, rinse well in tap water, and finally with distilled water, making sure that the perforations in the bottom of the crucibles are not clogged.

A synopsis of the technique is as follows:

1. Prepare the asbestos mat, $\frac{1}{16}$ inch thick.
2. Wash in distilled water.
3. Dry the crucible at 110°–120° C. for 15 minutes.
4. Ignite for five minutes.
5. Weigh. (No. 1.)
6. Affix crucible to filter flask and start suction pump.
7. Filter sample, decanting supernatant into crucible.
8. Wash residue with distilled water.
9. Dry for one hour at 110°–120° C.
10. Cool in desiccator.
11. Weigh. (No. 2.)
12. Ignite at low red heat in a radiator for 10 minutes.
13. Cool in desiccator.
14. Weigh. (No. 3.)

Weight No. 1=weight of crucible and mat.
Weight No. 2=ditto, plus suspended matter.
Weight No. 3=same as No. 2, minus volatile matter.

RELATIVE RAPIDITY AND ACCURACY OF THE GOOCH CRUCIBLE METHOD.

The determination of the solid matters in sewage and in water, as is well known, requires considerable care and involves a large expenditure of time. Where suspended matter results alone are desired, the advantages of a direct method are at once apparent. With the Gooch method the results under routine operation should be available in at least two hours.

That an idea may be had of the accuracy of the Gooch method for the determination of suspended matter in samples wherein the matters in suspension vary widely in amount and character, the following table has been prepared, which contains representative data for a large number of determinations.

TABLE 1.

REPRESENTATIVE DUPLICATE DETERMINATIONS OF SUSPENDED MATTER BY THE GOOCH CRUCIBLE.

NATURE OF SAMPLE	SUSPENDED MATTER, PARTS PER MILLION			
	Total		Volatile	
Crude sewage	183	184	106	100
" "	310	314	148	152
" "	130	134	95	96
Settled sewage	125	128	92	94
" "	214	217	103	105
" "	266	267	130	128
Effluents of coarse-grain filters	144	139	45	44
" " " " "	63	67	24	21
" " " " "	88	89	26	24
Average	169	171	85	87

ACCURACY AND RELATIVE ADVANTAGES OF THE GOOCH CRUCIBLE AND THE PLATINUM EVAPORATION METHODS.

Total suspended matter.—It is evident that the data obtained from the Gooch method will more correctly represent the actual weight of suspended matter, inasmuch as in all other methods the efficiency of sedimentation and of the filtration is not sufficient to include colloidal matter or substances in a very fine state of subdivision. It would therefore be expected that slightly higher results would be obtained by the Gooch method, were all the other factors associated with the indirect platinum method left out of consideration.

To illustrate the extent to which the colloidal character of the suspended matter affects the accuracy of the usual indirect platinum method, the data in the following table are given. In this table widely different samples of crude sewage were examined by the platinum method in two ways; first, after the usual filtration through paper, and second, after filtration through asbestos, as in the Gooch process. For the sake of comparison, the Gooch results were also included in the study.

The results show with considerable clearness the extent to which the efficiency of the filtration influences the results by the platinum method. The range of colloidal matter, as indicated from the data in the table below, is from 4 to 22, averaging 12 parts per million. From a practical standpoint it should be borne in mind that the platinum results are of equal value, since the suspended matter which is not included by the evaporation method forms a portion of that amount which cannot be removed by plain subsidence in an economical period of time.

TABLE 2.

REPRESENTATIVE RESULTS BY PLATINUM AND GOOCH METHODS, SHOWING THE EFFECT OF COLLOIDAL MATTER IN CRUDE SEWAGE.

SAMPLE OF CRUDE SEWAGE	SUSPENDED MATTER, PARTS PER MILLION			APPARENT COLLOIDAL MATTER, PARTS PER MILLION
	By Platinum Method Filtered through		By Gooch Method	
	Paper	Asbestos		
1	96	102	99	Max. 22
2	94	98	95	Min. 4
3	98	114	100	Average 12
4	72	86	96	
5	188	200	193	
6	170	192	180	
7	362	364	368	
8	196	210	223	

In the course of our investigations, a large number of determinations were made, comparing the Gooch and the platinum methods. The direct determination has always given higher results, ranging from 1 to about 33 per cent, and averaging about 13 per cent. As noted above, the completeness of the filtration seems to be one of the principal causes of these differences. The discrepancies are greatest where the suspended matters are low, and especially where they are more or less colloidal in character. Thus, in the table below it may be noted that the settled sprinkling filter effluents give the greatest variation, for in these the suspended matter is usually quite finely divided.

Without detracting from the value of the platinum results, which of course may be made more perfect by filtration through asbestos or a Berkefeld filter,[1] our experience indicates that the Gooch crucible method is of more practical applicability, owing to the relatively greater facility and speed with which suspended matter data may be obtained. Relative results by the two methods are shown in the following table:

TABLE 3.

Representative Relative Total Suspended Matter Results by Gooch and Platinum Methods.

Source of Sample	Suspended Matter, Parts per Million		Per Cent Which Gooch Results Are Higher than Platinum Results
	Gooch	Platinum	
Crude sewage	241	234	3
	636	632	1
	404	386	5
Settled sewage	186	164	12
	223	208	7
	103	78	24
Septic sewage	112	96	14
	146	122	16
	86	60	30
Coarse-grain filter	142	136	4
	211	196	7
	193	178	8
Settled effluents of coarse-grain filter	67	46	31
	89	70	21
	33	22	33
Average	....	....	13

THE INDIRECT DETERMINATION OF THE VOLATILE SUSPENDED MATTER.

It has been the practice for many years to ignite the dried and weighed residue upon evaporation of filtered and unfiltered samples, the loss in weight being taken to indicate the amount of organic matter present. The indirect determination of the volatile suspended matter is subject to a number of errors, as is well understood. In order that the residue shall be as free as possible from water of crystallization in the presence of incrustants, and to prevent to a considerable degree the loss of carbonic acid from magnesium salts also, and to convert all alkaline earth chlorides to the less volatile carbonates, it is customary to add a slight excess of sodium carbonate. Under such conditions, the residues do not usually contain water of crystallization, which will not be again taken up when the residues are moistened with water and evaporated. Owing to the complex and uncertain composition of the basic magnesium carbonate formed during the evaporation with sodium carbonate, considerable error is introduced upon ignition when magnesium is present, due to the loss of carbonic acid and some combined water.[1]

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[1] "Report of Committee on Standard Methods," *Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 44.

From the careful studies upon the "loss on ignition" made at Lawrence in 1890, it appears that the loss is approximately equal to 83 per cent of the weight of the magnesium (Mg) present. To determine further data upon this point, solutions of magnesium sulphate, in amount corresponding to 30, 60, 90, and 120 parts per million magnesium (Mg), were respectively treated with sodium carbonate in slight excess, evaporated in platinum, dried, and weighed. The dishes were then ignited for two minutes in the radiator, cooled, moistened with water, re-evaporated, dried, and weighed. Magnesium sulphate was used in this study for convenience. Since the magnesium of the alkalinity is also precipitated as a basic carbonate by heat and sodium carbonate, the end result of soda ash treatment is practically the same, whether or not, under natural conditions, incrustant magnesium is the only form in which the magnesium is present in the sewage.

From the results which appear in the table below, it is shown that the loss in weight, due to the loss of carbonic acid and combined water from the dried basic magnesium carbonate, is about 50 per cent of the total magnesium (Mg) present. As the average amount of magnesium (Mg) in the Columbus sewage is about 60 parts per million, the ignition of the residue of 50 c.c. of sewage entails a loss of 0.0015 gram, or 30 parts per million. These errors tend to balance each other in the unfiltered and filtered samples, so that the suspended loss is not affected to as marked a degree as might at first appear. The volatile matters, on the other hand, are obviously somewhat distorted.

TABLE 4.

LOSS IN WEIGHT UPON IGNITION OF MAGNESIUM SULPHATE AND SODIUM CARBONATE IN RADIATOR.
(Average of Two Experiments.)

Mg, Parts Per Million	Actual Weight Mg (Gram)	Actual Weight of Magnesium Sulphate and Sodium Carbonate	Loss in Weight after Two Min. Igni. (Gram)	Per Cent Loss in Weight
30	0.015	0.0146	0.0009	60
60	0.030	0.0285	0.0013	43
90	0.045	0.0413	0.0021	47
120	0.060	0.0556	0.0027	45
Average .	....	....	....	49

THE DIRECT DETERMINATION OF VOLATILE SUSPENDED MATTER BY THE GOOCH CRUCIBLE METHOD.

The evidence seems to be very clear that the Gooch crucible method furnishes data upon volatile suspended matter which more correctly represent the actual conditions, since in this method the actual loss in weight resulting from the burning of the organic constituents is measured by a primary process.

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[1] *Mass. State Board of Health Report*, 1890, 2, p. 715.

When experiments were first begun looking to the feasibility of the adoption of the Gooch crucible, attention was soon directed to the great discrepancies existing between the volatile matter results obtained by the Gooch, as compared with those given by the platinum method. The extent of the variability of volatile suspended matter results by the two methods may be noted from the results in the following table:

TABLE 5.

COMPARATIVE VOLATILE SUSPENDED MATTER RESULTS BY GOOCH AND PLATINUM METHODS.

SOURCE OF SAMPLE	VOLATILE SUSPENDED MATTER		
	Parts per Million		Per Cent Which Platinum Is of Gooch
	Gooch	Platinum	
Crude sewage	132	96	72
	205	28	63
	174	148	85
Settled sewage	107	70	65
	124	06	77
	65	32	49
Septic sewage	74	56	76
	70	40	51
	74	46	62
Effluent of coarse-grain filter	45	28	62
	50	30	60
	48	24	50
Settled effluent of coarse-grain filter	23	2	17
	25	4	16
	23	4	17
Average	83	49	59

FACTORS ASSOCIATED WITH THE VARIABLE RESULTS OF THE INDIRECT EVAPORATION METHOD AS EXPLANATORY OF THE DISCREPANCIES EXISTING BETWEEN THE GOOCH AND PLATINUM METHODS.

The results given in the preceding table, showing such wide variation in the volatile suspended matter figures for the same example, but examined by two different methods, suggest that one of them must be open to serious criticism. Since in the Gooch method we are dealing with the actual suspended matter, it would appear that the discrepancies must be looked for in the indirect method, which, as is well understood, is subject to many inherent sources of error when it becomes a question of the absolute significance of the results.[1]

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[1] *Mass. State Board of Health Report*, 1890, 2, p. 715.

One of the chief variables in the standard platinum method, which affects the indirect volatile suspended matter determination, refers to the incompleteness and uncertain character of the ignition. These irregularities have to do with the temperature of ignition, the time of ignition, and the relative amount and character of the mineral salts present. It is clear that the temperature of ignition must not be raised above the volatilization point of chlorides, nor above that at which oxygenated compounds become deoxygenated; and, as is well known, it is to avoid such complications that the radiator is used. Under such limitations it is apparent that the completeness of the combustion of the carbon will, in a large measure, depend upon its state of division, and perhaps upon the more important factor associated with the occluding property of the inorganic salts present. A close examination of residues ignited in platinum, according to the standard procedure, will show small particles of carbon remaining unoxidized. Since a complete ignition is understood to yield a residue white in color, save for the possible stain of oxide of iron, it is obvious that the present practice in regard to loss on ignition falls short of complete oxidation. Owing to the occlusion by the predominating mineral salts in residues from hard sewages, the loss on ignition results tend to be low, as already noted. To illustrate the

TABLE 6.

RELATIVE EFFECT OF SECOND IGNITION UPON THE TOTAL AND DISSOLVED VOLATILE MATTER.

CHARACTER OF SAMPLE	VOLATILE MATTER					
	Parts per Million				Per Cent Which First Ignition Is of Second Ignition	
	Total		Dissolved			
	1st Ignition	2d Ignition	1st Ignition	2d Ignition	Total	Dissolved
Crude sewage	72	92	44	54	78	81
	104	126	76	88	82	86
	98	106	78	82	91	95
Settled sewage	70	96	42	58	73	77
	178	200	114	122	89	93
	160	186	114	134	86	85
Septic sewage	74	110	40	58	67	70
	162	188	114	126	86	90
	182	208	126	156	88	81
Effluent of coarse-grain filter	68	98	40	52	70	77
	70	100	40	48	70	83
	66	108	40	50	61	80
Average	....	....	....	....	78	83

extent to which the occlusion of volatile matter by the mineral constituents of the residue is a factor in controlling the loss in weight on ignition, and also to illustrate what is thought to be one of the greatest sources of error in the determination of volatile suspended matter by the platinum method, the preceding table has been prepared, in which are presented data representative of a large number of determinations upon the point under discussion.

In the *Fourth Report of the Royal Bureau of Sewage Disposal and Water Purification* at Berlin,[1] in an account of the investigations at Cologne, Germany, the method used for the determination of the total and volatile suspended matter is given in detail. As refers to volatile matter, their method of ignition merits special comment, since it aims to ensure a very complete ignition, eliminating the occlusion factor to which we have already referred. In brief, the technique involves repeated ignitions, followed by applications of distilled water, until the small particles of carbon occluded by the mineral matter have entirely disappeared, leaving a pure white residue. Usually from four to six ignitions were necessary. To effect the ignition, the dish was moved cautiously back and forth over a free flame from a mushroom burner. As soon as considerable charring was noted, the residue was moistened with water, the water evaporated, and the ignition repeated as before. After the final ignition the residue was moistened with a few drops of ammonium carbonate solution, and then slightly warmed, in order to take up again the carbonic acid which might have been driven off in spite of the cautious heating.

Although, in the presence of magnesium, considerable error seems inevitable, yet this method appears to be an improvement over the ignition practices in this country, in which the occlusion of particles of organic matter by the mineral constituents of the residue is often a factor of considerable moment. To learn the extent to which the increased loss in weight could be attributed to the loss of carbonic acid from basic magnesium carbonate in the presence of sodium carbonate, a second ignition was made of the residues obtained in the magnesium experiments already described. It appears from these results that there may be an additional loss of about 8 per cent, or about five parts per million for 60 parts of magnesium (Mg), which is not to be traced to the more perfect combustion of the organic matter. Such errors as this obviously affect the total loss on ignition values rather than the volatile suspended matter results, as before mentioned. The effect of a second ignition upon basic magnesium carbonate is shown in the following table:

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[1] *Report of Royal Bureau of Sewage Disposal and Water Purification*, Berlin, Germany, 1904.

TABLE 7.

EFFECT OF SECOND IGNITION UPON BASIC MAGNESIUM CARBONATE.

(Mg) Parts per Million	Actual Weight Mg (Gram)	Loss in Total Weight (Gram)		Per Cent Total Loss in Weight	
		First Ignition	Second Ignition	First Ignition	Second Ignition
30.............	0.0150	0.0009	0.0010	60	67
60.............	0.0300	0.0014	0.0018	47	60
90.............	0.0450	0.0019	0.0023	43	51
120.............	0.0600	0.0023	0.0027	38	43
Average.....	....	....	....	47	55

To determine the effect of further treatment with water and a second ignition in the radiator, residues from the usual processes have been so treated. Upon the reapplication of distilled water, and as a result of the second ignition, we have obtained residues containing no visible particles of carbon. How far the elimination of the occlusion factor will increase the value of the suspended loss on ignition results, the following comparative data may serve to illustrate. These results also indicate, in a general way, that a second application of water and a reignition of the residue from the first ignition would greatly enhance the value of loss on ignition data, bearing in mind the magnesium factor already discussed.

TABLE 8.

RELATIVE RESULTS BY GOOCH AND PLATINUM METHODS SHOWING EFFECT OF SECOND IGNITION.

Character of Sample	Volatile Suspended Matter, Parts per Million		
	Platinum Method		Gooch Method
	Two Minute Ignition	Second Ignition Three Minutes	
Crude sewage............	28	38	43
" "	82	92	101
Settled sewage...........	28	38	44
" "	34	52	53
Effluent coarse-grain filter..................	30	52	50
	28	46	44
Settled effluent of coarse-grain filter............	4	28	28
	4	30	30

SUMMARY AND CONCLUSIONS.

The results of these studies seem to show, with considerable clearness, the practical applicability of the Gooch crucible to the determination of the amount and character of the suspended matter in sewages and in sewage effluents. The advantages of the Gooch crucible method consist in accuracy, speed, and completeness of filtration, and the comparatively small number of operations entailed in its technique. Compared with the usual platinum evaporation method, and aside from the admitted superiority of a direct process, the use of the Gooch crucible very materially reduces the time and labor involved in manipulation. It eliminates the errors of the evaporation method, relating to uncertain amounts of water of crystallization in the dried total and dissolved residues, respectively, and the liability to a variable loss during ignition, due to deoxygenating decomposition and volatilization of certain mineral salts. As indicated also, the Gooch method not only gives results the equal of those obtained by the platinum method, but, when desired, it affords a practical means for estimating the amount of very finely divided suspended matter usually contained in sewages.

In the case of the platinum evaporation method, our results suggest the strong advisability that a second ignition should be included in the standard procedure, both for sewage and for polluted water, since by this means a more nearly perfect combustion of the organic matter seems assured at radiator temperature. An initial partial ignition of the residue, followed by the application of a small amount of distilled water, evaporation, and a second ignition, it is thought, would ensure a very complete oxidation of the organic matters at a low radiator temperature, thus obtaining the most efficient result at a temperature far below the volatilization point of mineral matter. Further, it is considered that asbestos could be used to advantage as a filtering material in sewage work, in order to effect a more complete removal of finely divided suspended matter than is possible by the use of filter paper.

During the investigations at Columbus, over 500 determinations of suspended matter were made by the Gooch crucible method, in samples ranging from the crude sewage containing as high as 1,500 parts per million of total suspended matter, to settled effluents from sprinkling filters containing as low as 20 parts per million of suspended matter, duplicate results showing that the method was at least applicable to this range of suspended matter in amount and character.

Such being the range of the applicability of the Gooch crucible method and in view of the several advantages it possesses over the indirect platinum method for the determination of suspended matters, the conclusion seems justified that, for practical routine work upon sewage, especially in investigations relating to physical questions involved in sewage purification, the Gooch crucible method deserves recognition as a standard method.

In conclusion the writers desire to express their thanks to Mr. Julian Griggs, chief engineer of the Board of Public Service, Columbus, Ohio, through whose courtesy they have been permitted to publish the results of these studies in advance of the regular testing-station report. Further, they desire heartily to acknowledge the valuable criticisms of Mr. George W. Fuller, consulting engineer, and the helpful suggestions of Mr. George A. Johnson, engineer in charge of the Columbus Testing Station.

THE RESISTANCE TO DECOMPOSITION OF CERTAIN ORGANIC MATTERS IN SEWAGE.

H. W. CLARK.

THIS investigation, in regard to the resistance to decomposition of certain organic matters, was begun in order thoroughly to comprehend the conditions prevailing in certain sand filters which had received sewage for periods varying from 10 to 17 years. Studies of the work accomplished by these filters during these many years of operation show that only about 55 per cent, of the nitrogen in the sewage applied to them has appeared in their effluents. Studies of municipal areas, moreover, receiving a fresher sewage, have shown that only about 30 per cent of the applied nitrogen appears in the effluents from these areas. Much of the remaining nitrogen is set free by nitrogen-liberating bacteria; but a small percentage of the nitrogenous matters is resistant to bacterial actions and accumulates within the filter—from 4 to 8 per cent in the experimental filters at Lawrence.[1]

The filters under discussion had stored, in the period stated, enough organic matter seriously to impair their satisfactory operation; that is, the upper layers of these filters had become clogged to such an extent that it was necessary to break through these layers and to ridge and trench the surface of the filters in order that good purification of the applied sewage might be obtained. It was hoped that, by this surface treatment, the sand piled in ridges would lose the larger part of its stored organic matters by the work of the bacteria present, thus given a chance to work over the organic matter stored in the sand, rather than the organic matter in the sewage applied daily.

From the sand of the ridges, arranged in this way upon the three filters chiefly studied, differing amounts of nitrogen disappeared. From the ridges upon two filters constructed of coarse sand, 46 per cent of the stored nitrogen disappeared in three months, and from the ridges upon a filter constructed of comparatively fine sand, only about 10 per cent disappeared in an equal period. The residual nitrogenous matters remaining upon the sand in these ridges remained, however, after this first quick and easy removal, month after month without change, during weather favorable to bacterial activity in the filters.

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[1] For a fuller account of this work, see *Report of Massachusetts State Board of Health*, 1904.

Following this work, small filters of sand, taken from the upper portions of the three sand filters under investigation and rich in organic matter, were constructed. These small filters, started early in May, 1904, were placed in the laboratory and kept under conditions favorable to nitrification. Each of these filters was flooded with water, and the rate of operation of each was 30,000 gallons per acre daily. Nitrification started immediately in the filter constructed of coarse sand, and at the end of a week of operation, the effluent of this filter contained 15 per cent of nitrates per 100,000. In the other two filters, nitrification also started quickly, and the nitrates in the effluent of each became comparatively high very soon after the beginning of their operation. The nitrates remained high for about two months, and much of the nitrogenous organic matter on the sand was removed during this period; from the filter of coarse sand, 70 per cent, and from the remaining filters 55 and 30 per cent, respectively. At the end of this period of quick nitrification, however, although conditions were the same, nitrification practically ceased, and the organic matter upon the sand, both nitrogenous and carbonaceous, remained constant in amount. Determinations of the nitrogen in each filter and in the effluent of each were made, and it was shown that of the total amount of nitrogen removed from the sand of each filter during its period of operation a very varying amount had passed off in the three effluents; in the effluent of one, only 18 per cent of the total amount, in the effluent of the second, 23 per cent, and in the effluent of the third, 71 per cent. That is to say, much nitrogen lost from the sand passed away in the air, as the result of the work of the nitrogen-liberating bacteria undoubtedly present in each filter, the largest amount being liberated from the filter of coarsest sand.

After nitrification had been low in each filter for a period of several months, and the organic matter upon the sand had remained at a practically constant figure, as shown by various analyses, attempts were made to increase nitrification, by dosing each filter with chemicals or cultures of bacteria, to induce the activity of the nitrifying organisms. Small amounts of sewage were added, also, in order to be sure that nitrifying bacteria were present in each filter, but this was without effect in increasing nitrification. Further, in order to prove that the filters were capable of producing nitrates, if easily nitrified matter was applied to them, a solution of ammonium chloride and sodium carbonate was applied to one filter, and peptone to a second filter. Following the application of these substances, nitrification again became high in each filter, but ceased quickly when these bodies were omitted from the water applied.

In order to study further the character of the matter stored in these filters, determinations of the total organic matter present were made. By this means, it was found that the nitrogen present was a very small and varying per cent of the total organic matter, probably not more than 3 per cent, judging from many analyses, and that the principal clogging matter upon the sand was carbonaceous and of the nature of cellulose. The investigation is still under way, but so far shows clearly that there is in ordinary domestic sewage a certain small percentage of exceedingly stable carbonaceous and nitrogenous matter, that, accumulating year after year, seriously impairs the work of these filters; that these matters are but slowly affected by the ordinary bacterial actions depended upon to oxidize, or cause the disappearance, of organic matter; that even when this clogged sand is taken from the filters and placed under conditions favorable to nitrification, as in the small filters described in this paper, a considerable portion of the organic matter remains unaffected; that the larger part of this matter is of a carbonaceous rather than of a nitrogenous nature. The practical bearing of this is that it indicates clearly that from the ordinary intermittent sand filter for the disposal of sewage, sand will, in most instances, eventually have to be removed, notwithstanding careful oversight and good operation of such filters.

THE COLLECTION AND PRESERVATION OF SAMPLES OF SEWAGE FOR ANALYSIS.

Stephen DeM. Gage and George O. Adams.

It is well known that the composition of the sewage from any source is not uniform, varying with the time of day, the amount of surface water entering the sewers, and also with the temperature and with the time which elapses between collection and analysis. The variations in the composition due to these different factors have been frequently discussed, and it is unnecessary to enter into their further consideration at this time.

In the design and control of sewage disposal systems it is of the first importance to obtain the true average composition of each day's flow of sewage, by collecting series of samples covering 24 hour periods. The number of such samples which may be collected is usually limited, however, by the capacity of the testing laboratory to make the necessary analytical determinations before decomposition has started, and also unless exceptional facilities for the transportation of samples are provided, the time elapsing between collection and analysis is sufficient in many cases to affect seriously the accuracy of the results. If some simple and effective method of preserving these samples were used, the capacity of the laboratory to handle a series of samples would be greatly increased, as the work could be extended over several days; and there would also be an increase in the value of the analytical results, since the samples when analyzed would be in the same condition as when collected.

In the control of many municipal sewage areas it is customary to send samples to a central laboratory at stated times, and from the results of the analyses of these samples to interpret the purification accomplished by the area. That these samples are not representative is well known, but hitherto attention has been centered on improving the analytical methods, to the neglect of methods of sampling. Monthly or semi-monthly analyses of average samples, made up of small portions collected two or three times daily and preserved in some manner by the filter attendants, would yield more representative results without imposing any great burden on the filter attendants.

Two methods of preserving samples are open to us: the use of cold and the use of chemicals. The use of cold, while fairly satisfactory for short periods, is limited by the fact that ice is not readily obtainable at sewage disposal works, is difficult to handle, increases transportation charges, and even when obtainable is less convenient than chemical treatment. It is generally understood that the decomposition which takes place in sewage and in the effluents from sewage filters is largely, if not entirely, due to bacteriolytic action. To be thoroughly effective, then, the chemical employed as a preservative should immediately check all bacterial action, should be comparatively cheap and easily obtainable, and, above all, should not interfere with any of the chemical determinations.

The report of the Committee on Standard Methods of Water Analysis,[1] discussing the time which may be allowed to elapse between collection and analysis of samples, states (p. 14) that six hours is the maximum which may be permitted for sewages and the effluents from sewage purification systems. On p. 15, the report says: "If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other disinfectant, samples for chemical and microscopical examination may be allowed to stand for longer periods. . . . ."

Neither formaldehyde or mercuric chloride conforms to the requirements of an ideal sewage preservative, in that both interfere with certain of the chemical determinations, formaldehyde giving a yellow color with Nessler reagent in the free ammonia determinations and reducing the permanganate of potash in the oxygen consumed process, and mercuric chloride interfering with the determinations of both chlorine and solids. For many years, mercuric acetate has been used at the Lawrence Experiment Station for preserving average samples of sewage; but while the disinfecting action of this has been entirely satisfactory, it is not readily obtained, and interferes with the determination of the solids.

As a result of studies made at the Lawrence Experiment Station during the past year, it has been found that chloroform and carbon-bisulphide most nearly fulfil the ideal conditions. Both are readily obtainable, are sparingly soluble in water, and, being heavier than water, do not evaporate rapidly, requiring only a small amount in each sample. Chloroform, however, is the more satisfactory of the two, being more pleasant to use, thoroughly effective, and, so far as

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[1] *Jour. Infect. Dis.*, 1905, Supplm. No. 1 p. 1.

TABLE I.

THE RESULTS OF CHEMICAL DETERMINATIONS ON DUPLICATE SAMPLES WITH AND WITHOUT THE PRESENCE OF CHLOROFORM.

	FREE AMMONIA		ALBUMINOID AMMONIA	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter........	0.0434	0.0426	0.0220	0.0220
" " "	0.9750	0.9500	0.1460	0.1340
Effluent trickling filter.....	0.1120	0.1040	0.0960	0.1020
Effluent contact filter......	1.0000	1.0800	0.1480	0.1360
" " "	0.6600	0.6800	0.1500	0.1260
Effluent septic tank.......	2.2000	2.2000	0.1900	0.2300
Raw sewage.............	5.3000	5.1000	0.4400	0.4300

NITROGEN AS

	NITRATES		NITRITES	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter........	2.94	2.94	0.0032	0.0032
" " "	2.94	3.11	0.0028	0.0032
Effluent trickling filter.....	1.51	1.60	0.0040	0.0040
" " "	1.43	1.60	0.0020	0.0020
Effluent contact filter......	0.02	0.02	0.0000	0.0000
" " "	1.03	1.07	......	......

	CHLORINE		OXYGEN CONSUMED	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter........	10.20	10.20	0.37	0.37
Effluent trickling filter.....	8.42	8.42	1.51	1.60
" " "	7.50	7.50	1.58	1.18
Effluent contact filter......	10.58	10.58	1.20	1.26
" " "	9.60	9.60	0.82	0.74
Effluent septic tank.......	8.80	8.80	3.64	3.60
" " "	9.20	9.20	1.12	1.30
Raw sewage.....	10.10	10.10	5.45	5.70

ORGANIC NITROGEN (KJELDAHL).

	With Chloroform	Without Chloroform
Effluent sand filter.........	0.2698	0.2936
" " "	0.2936	0.2558
Effluent trickling filter......	0.1788	0.1779
" " "	0.1878	0.1779
Effluent contact filter.......	0.2731	0.2772
" " "	0.2706	0.2501
Effluent septic tank.........	0.2854	0.2837
Raw sewage..............	0.4400	0.4300

we have been able to determine, not interfering with any of the chemical determinations. Carbon-bisulphide interferes with the determination of nitrates by the aluminum method, and is decomposed by some sewages into hydrogen sulphide or free sulphur, which interferes with some of the chemical determinations, and renders it less effective in preventing bacterial decomposition. In order to prove that chloroform had no effect on the results of the various analytical determinations, many determinations were made of samples with and without the addition of that substance. Some of these comparative determinations are shown in Table 1, from which it is seen that, while the results vary slightly among themselves, they are well within the limits of error of sampling and analysis.

Experiments were then made to determine the proportion of chloroform necessary to destroy the bacteria, or at least to prevent them from multiplying. Various amounts of chloroform were added to bottles of sewage from which the suspended matter had been removed by filtering through paper, and the samples were allowed to stand in the laboratory, determinations of the numbers of bacteria being made at frequent intervals. In these experiments it was found that between 5 and 10 c.c. of chloroform in one gallon of sewage would control the bacteria, this amount making practically a saturated solution. In practice based on further experiments, it has been found advisable to add somewhat more chloroform, and to recover the excess when the analyses are complete. The recovery is easily

TABLE 2.

THE EFFECT OF DIFFERENT AMOUNTS OF CHLOROFORM ON THE BACTERIA IN ONE GALLON OF SEWAGE.
(Bacteria per c.c.)

Elapsed Time	No Chloroform	2 c.c. of Chloroform	5 c.c. of Chloroform	10 c.c. of Chloroform
Start	1,270,000	1,300,000	370,000	10,000
1 day	1,650,000	760,000	3,000	300
2 days	2,200,000	1,160,000	6,500	16
3 “	650,000	1,140,000	75	16
4 “	900,000	1,690,000	2,000	90
5 “	730,000	2,000,000	3,900	75
7 “	830,000	3,050,000	1,600	60
9 “	820,000	7,200,000	22,400	180
11 “	480,000	5,250,000	140	30
15 “	190,000	3,120,000	5,800	5
18 “	80,600	6,840,000	6,800	30
21 “	500,000	10,800,000	108,000	90
28 “	280,000	15,120,000	4,600	100
35 “	380,000	21,650,000	90	42
42 “	14,000	6,770,000	28,600	32
49 “	71,400	14,040,000	19,200	75
63 “	24,500	2,250,000	280	60

made by inverting the samples over a funnel in another bottle filled with water, when the chloroform sinks into the lower bottle, and may be distilled when a sufficient amount has accumulated. The effectiveness of different proportions of chloroform in controlling bacterial life is shown in Table 2.

As a method of preservation would, in practical use, be subjected to considerable variation in temperature, experiments were made to determine the effectiveness of chloroform in preserving samples which were allowed to stand at different temperatures. Samples of sewage, from which the suspended matter had been removed by filtering through paper, were treated with chloroform and incubated at 10°, 20°, and 30° C., respectively, analyses being made at frequent intervals. The results of the various determinations fluctuated more or less, but no regular change appears to have taken place in any of the samples, and it is probable that the fluctuations were due to errors in obtaining the proper sample from the bottle and in the chemical methods. The most noticeable change was in the oxygen consumed, and part of this may have been due to oxidation during the numerous shakings incident to removing samples for 13 analyses. The various analytical results in one experiment with sewage treated with chloroform standing at different temperatures are shown in Tables 3, 4, and 5.

In order to test the applicability of the method to sewages which had been more or less completely purified, experiments were made in

TABLE 3.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 10° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start	5.10	0.44	0.82	43.5	5.45	39,000
1 day	5.30	0.43	0.81	43.6	5.70	0
2 days	4.50	0.43	0.78	45.3	4.60	24
3 “	5.60	0.46	0.74	51.1	5.45	16
4 “	5.60	0.45	0.92	40.1	4.60	3
5 “	5.00	0.44	0.82	44.0	5.30	70
7 “	4.90	0.43	0.78	45.3	4.70	22
10 “	5.20	0.39	0.91	35.2	4.85	41
15 “	4.70	0.42	0.74	46.5	4.75	10
17 “	5.20	0.47	0.84	45.8	4.20	14
22 “	4.80	0.38	0.83	39.5	4.65	16
28 “	4.80	0.42	0.83	35.5	4.85	27
36 “	5.00	0.39	0.67	47.8	4.30	25
42 “	5.70	0.41	0.74	45.4	4.25	25

TABLE 4.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 20° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start	5.10	0.44	0.82	43.5	5.45	39,000
1 day	4.70	0.42	0.81	42.5	5.20	200
2 days	4.60	0.41	0.73	46.0	4.45	26
3 "	5.00	0.45	0.74	50.0	5.35	17
4 "	5.30	0.50	0.94	43.6	4.45	11
5 "	5.50	0.43	0.81	43.6	3.55	20
7 "	5.20	0.45	0.81	45.6	4.60	25
10 "	5.20	0.43	....	....	4.50	28
15 "	5.30	0.41	0.81	41.5	4.60	15
17 "	5.20	0.42	0.85	41.0	4.00	6
22 "	4.80	0.40	0.75	45.9	4.30	20
28 "	4.70	0.44	0.78	46.3	4.55	21
36 "	5.30	0.42	0.68	50.6	4.20	55
42 "	5.20	0.41	0.84	40.0	4.40	28

TABLE 5.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 30° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start	5.10	0.44	0.82	43.5	5.45	39,000
1 day	5.50	0.45	0.82	45.0	4.45	300
2 days	5.10	0.39	0.79	40.7	3.95	26
3 "	4.70	0.42	0.72	47.9	4.30	24
4 "	5.40	0.48	0.84	46.9	4.30	23
5 "	6.20	0.43	0.82	43.1	2.50	30
7 "	5.20	0.40	0.75	43.7	4.30	23
10 "	5.20	0.40	0.88	37.3	4.40	40
15 "	4.90	0.41	0.77	43.6	4.35	2
17 "	4.40	0.48	0.83	47.5	3.75	8
22 "	5.30	0.42	0.89	38.7	3.95	16
28 "	5.60	0.41	0.74	45.4	4.30	17
36 "	5.20	0.44	0.84	43.0	4.00	26
42 "	5.20	0.41	0.78	48.1	4.15	31

which effluents from a sand filter, a trickling filter, a contact filter, and septic tank were treated with an excess of chloroform, to determine whether the destruction of the bacteria by the antiseptic would prevent chemical decomposition, as had been noted in the raw sewages. The same fluctuations occurred in these samples as were previously noted with sewage samples. The variations, however, were greater than in the sewages above, because while the former contained little suspended matter, there was a considerable amount in the latter, making it more difficult to obtain a true sample. The results are shown in the following table:

TABLE 6.

Table Showing Preservative Action of Chloroform on Septic Sewage, and the Effluents from Sand, Contact, and Trickling Filters.

Elapsed Time	Ammonia		Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Nitrogen as		Bacteria per c.c.
	Free	Alb.				Nitrates	Nitrites	
				EFFLUENT SAND FILTER.				
Start........	0.9500	0.1460	0.2936	40.8	0.42	1.48	0.2000	72,000
4 days......	0.9750	0.1340	0.2558	42.9	0.96	1.81	0.0000	108,000
8 "	0.8750	0.1400	0.2378	48.3	1.01	1.77	0.0000	200
15 "	0.8400	0.1460	0.2608	45.9	1.05	0.91	0.0600	600
22 "	0.7600	0.1740	0.2698	52.3	1.00	1.18	0.0000	98
32 "	......	......	......	....	....	1.68	0.0000	....
				EFFLUENT SAND FILTER.				
Start........	0.0426	0.0220	......	....	0.37	2.94	0.0032	1,900
3 days......	......	......	......	....	0.39	3.11	0.0028	14
10 "	......	......	......	....	0.30	2.69	0.0030	....
17 "	0.0378	0.0208	......	....	0.39	3.11	0.0028	450
				EFFLUENT TRICKLING FILTER.				
Start........	0.1760	0.1880	0.3378	45.6	1.33	0.91	0.0130	80,000
4 days......	0.0880	0.2040	0.4478	37.4	1.14	1.09	0.0050	76,000
8 "	0.0880	0.2160	0.3649	48.4	1.28	0.92	0.0006	1,000
15 "	0.1120	0.2160	0.4264	41.6	1.61	0.88	0.0050	1,170
22 "	0.1120	0.2200	0.3813	47.3	1.36	1.01	0.0020	720
32 "	......	......	......	....	....	1.09	0.0014	....
				EFFLUENT TRICKLING FILTER.				
Start........	0.1040	0.1020	0.1779	47.0	1.18	1.60	0.0040	17,500
3 days......	0.1120	0.0960	0.1878	49.8	1.58	1.43	0.0020	30,000
10 "	0.1320	0.1220	0.1911	52.4	1.36	1.55	0.0032	640
17 "	0.1340	0.1320	0.2075	52.1	1.32	1.48	0.0032	800
				EFFLUENT CONTACT FILTER.				
Start........	0.6800	0.1260	0.2501	41.3	0.74	1.07	0.1900	650,000
4 days......	0.6600	0.1500	0.2706	45.5	0.82	1.03	0.1500	165,000
8 "	0.5400	0.1720	0.2919	48.3	0.85	0.89	0.0400	26
15 "	0.6400	0.1640	0.2583	52.0	0.87	0.72	0.0800	22
22 "	0.6400	0.1720	0.3050	46.2	0.84	0.84	0.0400	14
32 "	......	......	......	....	....	0.95	0.0400	....
				EFFLUENT SEPTIC TANK.				
Start........	2.20	0.23	0.42	45.0	1.30	....	......	80,000
4 days......	2.20	0.19	0.34	45.9	1.12	....	......	120,000
8 "	2.10	0.17	0.31	44.3	1.30	....	......	85
15 "	1.90	0.20	0.30	55.2	1.44	....	......	119
22 "	2.00	0.17	0.30	47.0	1.14	....	......	143

In the foregoing the most marked percentage change noted was in the nitrites. In order to study these nitrite changes, further experiments were made in which mixtures of sewage and water containing sodium nitrite were treated with chloroform. In these experiments the nitrites in the samples treated with chloroform were unchanged, which would seem to show that the changes occurring in the nitrites in the effluents noted above were due to chemical, rather than bacterial, action, a view recently advanced by Phelps[1] as the result of his studies of the action of contact filters. The results of one of these experiments are shown in the following table:

TABLE 7.

NITRITES.

	CONTROL		CHLOROFORM	
Elapsed Time	Parts per 100,000	Bacteria per c.c.	Parts per 100,000	Bacteria per c.c.
Start............	0.0320	30,000	0.0320	30,000
2 days...........	0.0320	86,400	0.0320	55
5 "	0.0040	4,430,000	0.0320	18
9 "	0.0000	5,260,000	0.0320	27
12 "	0.0000	2,710,000	0.0320	50
16 "	0.0000	5,250,000	0.0320	19

It has been frequently stated in the preceding that the fluctuations in the analytical results were within the errors of sampling and analysis. In order to ascertain what these errors would be in actual practice, 10 complete analyses were made of a sample of Lawrence sewage, and 10 determinations of nitrates and nitrites were made on the effluent from a trickling filter which contained a considerable amount of suspended matter. In making these analyses the routine procedure was followed, the work being divided between two analysts. The possible errors are from two sources; those due to sampling, i. e., the error in measuring out the volume for each determination and in obtaining an aliquot portion of the suspended matter in the sample, and those existing in the analytical methods. The sampling errors are variable for the different determinations, since it is necessary to take different volumes of the sewage. In the determination of free and unfiltered albuminoid ammonia in this experiment 5 c.c. of the sewage were used, and, owing to the large amount of suspended matter, it was necessary to measure that volume in cylinders, which are less accurate than flasks or pipettes. In determining the filtered albuminoid ammonia 10 c.c. of the sewage were used, the measurements being made also in cylinders.

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[1] "Contributions from the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology," *Jour. Infect. Dis.* 1905, Supplm. No. 1, p. 61.

The volumes of sewage for the determination of organic nitrogen by the Kjeldahl method were measured in flasks, 20 c.c. of the unfiltered and 25 c.c. of the filtered sewage being taken. Samples of the sewage for oxygen consumed were measured in 25 c.c. flasks, and for chlorine in 50 c.c. flasks. In the determination of nitrates 100 c.c. were measured in a flask, boiled down to 25 c.c., made up to 50 c.c. in a Nessler tube, and 2 c.c. of this, measured in a pipette, diluted to 50 c.c. in a Nessler tube for reading. The nitrite determinations were made on 10 c.c., measured in a pipette and diluted to 50 c.c. in a Nessler tube.

The error in the free ammonia as determined by the average deviation from the mean of the 10 determinations was 5 per cent. The mean error in the albuminoid ammonia determinations was 8 per cent and 8.5 per cent, respectively, for unfiltered and filtered samples. For organic nitrogen determined by the Kjeldahl process, the average deviation from the mean was 2.5 per cent for unfiltered samples and 3.3 per cent for filtered samples. The error of the chlorine determinations was practically negligible, while that of the oxygen consumed process was 2.1 per cent. There was no error in the nitrite determinations, all of the 10 results being identical. The

TABLE 8.

THE VARIATION IN RESULTS OF 10 SEPARATE CHEMICAL ANALYSES.

	SEWAGE							TRICKLING FILTER EFFLUENT	
	FREE AMMONIA	ALBUMINOID AMMONIA		ORGANIC NITROGEN (KJELDAHL)		CHLORINE	OXYGEN CONSUMED	NITROGEN AS,	
		Unfiltered	Filtered	Unfiltered	Filtered			Nitrates	Nitrites
1	6.20	0.86	0.46	1.95	0.89	11.80	4.88	1.00	0.0080
2	6.70	0.98	0.53	1.98	0.95	11.90	4.92	1.15	0.0080
3	7.00	0.98	0.43	2.02	0.85	11.89	4.76	1.19	0.0080
4	6.80	1.04	0.43	2.01	0.87	11.91	4.68	1.09	0.0080
5	7.10	1.12	0.39	1.93	0.93	11.80	4.60	1.09	0.0080
6	7.20	1.14	0.41	1.94	0.93	11.85	4.72	1.11	0.0080
7	6.80	1.12	0.51	2.07	0.99	11.80	4.56	1.07	0.0080
8	5.70	1.06	0.53	1.92	0.92	11.80	4.64	1.09	0.0080
9	7.00	0.92	0.47	1.82	0.92	11.80	4.68	1.09	0.0080
10	6.30	0.92	0.50	2.02	0.92	11.80	4.52	1.07	0.0080
Average	6.68	1.01	0.47	1.97	0.92	11.84	4.70	1.10	0.0080
Maximum	7.20	1.14	0.53	2.07	0.99	11.91	4.92	1.19	0.0080
Minimum	5.70	0.86	0.39	1.82	0.85	11.80	4.52	1.00	0.0080

average deviation of the nitrate results from the mean of the 10 readings was 3.6 per cent. The results of the 10 complete analyses of the sewage and 10 determinations for nitrates and nitrites on the trickling filter effluent are shown in Table 8.

CONCLUSIONS.

It is well known that considerable changes may take place in the composition of sewage and the effluents from sewage filters during short periods, and that the elapsing of a few hours between the time of collection and analysis may seriously affect the results of the analyses. Furthermore, samples collected at occasional intervals from sewers or from the effluents of sewage disposal works do not represent the actual average composition of waters from these sources. To be of the greatest value, samples should be collected at frequent intervals, preserved in some manner to prevent chemical changes, and then mixed in aliquot portions to form an average sample for analysis. Experiments at the Lawrence Station have shown that chloroform is a good preservative to use under these circumstances. From 10 to 25 c.c. of chloroform may be added to a gallon bottle, and small samples of from 100 to 200 c.c., collected daily, may be placed in the bottle, which is tightly stoppered; the average sample so obtained may be analyzed after any stated period without fear of material change in the individual samples of which it is composed. The principle errors which may result from such treatment are a small change in the nitrites, and the difficulty arising from the settling out of suspended matter. The nitrite difficulty may be obviated by taking occasional samples and doing nitrite determinations only. Any objection as to precipitation while the average sample is being collected affecting the determination of soluble and suspended organic matter may be overcome by filtering and preserving a separate portion of the daily sample. If such methods be carried out in practice, it is believed that they will result in a more accurate knowledge of the composition of applied sewages and the effluents from sewage disposal works, and will yield more accurate information as to the purification accomplished by the different methods of sewage disposal.

A READY METHOD OF PREPARING A SILICA TURBIDITY STANDARD.

ERNEST C. LEVY,
Director of Laboratory of City Water Department, Richmond, Va.

ALTHOUGH the candle or electric light turbidimeter has largely replaced comparison with silica standards as a laboratory method of determining the turbidity of water, these instruments, equipped with the usual short tube, are of use only where the turbidity is above 100, while the platinum wire method is applicable only in field work. Even there its use is limited to waters of neither very high nor very low turbidity, besides which there is not infrequently great difficulty in securing the necessary conditions of light. Direct comparison with silica standards is, therefore, the method which must be resorted to in most cases where the turbidity is below 100.

Of all standards used by the water analyst, the silica turbidity standard is the most difficult to prepare. Even after the very tedious preparation of the diatomaceous silica itself, the powder so obtained is not always of the required degree of fineness, thus necessitating standardization by the platinum wire method or by use of the candle turbidimeter, instead of simply adding a gram per liter as originally recommended, to secure a standard of 1,000 parts turbidity per million.

The above considerations led the writer, about a year ago, to examine a number of commercial products with the view of obtaining a satisfactory substitute for the diatomaceous silica, so difficult of preparation. Various polishing powders, advertised by the manufacturers as being made from diatomaceous earth, were first tried, but none of these possessed the requisite degree of fineness or uniformity. Finally a test was made of a toilet preparation sold under the name of "Pears' Precipitated Fuller's Earth," and this has appeared to meet all the practical requirements.

This powder is almost pure white in appearance, and standards prepared from it are indistinguishable from those made from diatomaceous silica secured by the method recommended originally by

Whipple and Jackson and incorporated in the "Report of the Committee on Standard Methods of Water Analysis," of this Association.[1] It has even seemed to the writer that the tendency to striation, noticeable chiefly in the higher standards, is less marked.

A rough analysis of the "precipitated fuller's earth" showed it to be largely a silicate of aluminum, rather than a true silica, and the term "precipitated" is probably not used in its chemical sense. The theoretical objection of its not being a strict silica does not seem to the writer to be a serious objection, since the powder shows no tendency to lump on standing, and standards made five months ago still maintain their original turbidity unaltered.

A request to the manufacturers, asking certain particulars concerning this preparation, especially as to whether the method of manufacture was such that uniformity of the product could be assured, brought no response, but several lots bought at different times were all perfectly uniform. As each package contains about 200 grams, gross, it is a simple matter to standardize each lot when purchased, and thereafter standards can be made quickly at any time. The writer has found, however, that a suspension of one gram per liter gives a standard of 1,000 in all of the samples tested; and it would therefore seem probable that this would regularly be the case, though, naturally, it would be advisable not to assume this to be so without actual testing of each new lot.

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[1] *Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 1.

THE SOLUBILITY OF CALCIUM CARBONATE AND OF MAGNESIUM HYDROXIDE AND THE PRÉCIPITATION OF THESE SALTS WITH LIME WATER.

GEORGE C. WHIPPLE AND ANDREW MAYER, JR.

THE problem of water softening is attaining such great importance that it seems advisable at this time to re-examine carefully the principles and chemical data involved, in order to render them as correct and trustworthy as possible. Prominent among the questions to be settled are the solubilities of normal calcium carbonate and of magnesium hydroxide.

SOLUBILITY OF NORMAL CALCIUM CARBONATE.

The values given for the solubility of calcium carbonate by the different authorities quoted in Comey's *Dictionary of Chemical Solubilities*[1] vary from 10 to 113 parts per million. About one-half of these authorities give values of more than 50 parts per million, but the high figures represent chiefly the results of early experiments, and are evidently erroneous. The figures which appear to be most reasonable may be divided into two groups, one placing the solubility between 20 and 36 parts per million, the other, between 10 and 16 parts per million.

TABLE I.

GROUP I.

Authority	Date	Temperature ° C.	Solubility in Parts per Million
Chevalet[2]	1869	..	34
Hoffman[3]	1865	..	34
Peligot[4]	....	..	20
Weltzien[5]	1865	..	36

GROUP II.

Authority	Date	Temperature ° C.	Solubility in Parts per Million
Bineau[6]	1857	..	16.0
Schlösing[7]	1872	16.0	13.1
Holleman[8]	1893	8.7	10.0
Holleman[8]	1893	23.8	12.5
Kohlrausch and Rose[9]	1893	18.0	13.0

Various methods were employed by the different investigators to secure these results, as indicated in the subjoined notes. Those who obtained values greater than 30 parts per million did not mention having taken any precaution against two of the greatest sources of error involved, namely, the influence of the CO_2 of the air, and the action of the solution of $CaCO_3$ upon the glass vessels. It seems significant that the figures of Bineau, who apparently first made allowance for these sources of error, and those of the modern experimenters, who followed his example, are all well under 20. The value most commonly accepted for the solubility of the normal calcium carbonate is 30 parts per million, although Kimberly, in a recent paper,[10] used 20 parts per million as the best value.

Several preliminary experiments to determine the solubility of normal calcium carbonate were conducted by one of us, in 1903. The pure salt was boiled with distilled water, free from CO_2, in flasks of Bohemian glass, and after standing for some days, protected from the CO_2 of the air, the resulting solution was filtered and titrated with N/50 acid. The results so obtained were 30.5 parts per million by one set of experiments, and 25 parts per million by another. During the following year these experiments were repeated, giving in one case 23 parts per million and in another 13.5 parts per million. The last value was obtained when a flask of Jena glass was substituted for the Bohemian glass flasks.

In order to determine the reason for these discrepancies and to obtain correct and concordant results, the following experiments were performed:

Two liter flasks of Jena glass were each fitted with a one-hole rubber stopper, through which projected a short piece of glass tubing, drawn to capillary dimensions at its upper end. Into each flask was put about 900 c.c. of distilled water, absolutely neutral in reaction to methyl orange, and lacmoid. The contents of the flasks were boiled for two hours, in order to drive off the last trace of CO_2 gas, and at the end of this time the stoppers were removed, and replaced, as quickly as possible, before any air could enter, by one-hole stoppers each bearing a U tube filled with beads, well moistened with fresh concentrated NaOH solution, the bend of the tube forming an effective seal. Flask 1 was cooled to 37° C. by allowing it to stand over night in an incubator adjusted to maintain that temperature, while Flask 2 was cooled to room temperature (21° C.). There was then quickly introduced into each flask 5 g. $CaCO_3$, prepared by thoroughly washing the best procurable chemically pure product with hot distilled water, and drying to constant weight at 100°–110° C. After stoppering with solid rubber stoppers, the flasks were kept, respectively, at the temperatures mentioned, and were frequently well shaken throughout the experiment, in order to render solution complete. At intervals of 24 hours portions were withdrawn, quickly filtered through paper, and 100 c.c. of the filtrate titrated with N/50 H_2SO_4, using phenolphthalein and methyl orange as indicators. The results are shown in the following table:

TABLE 2.

FLASK 1.

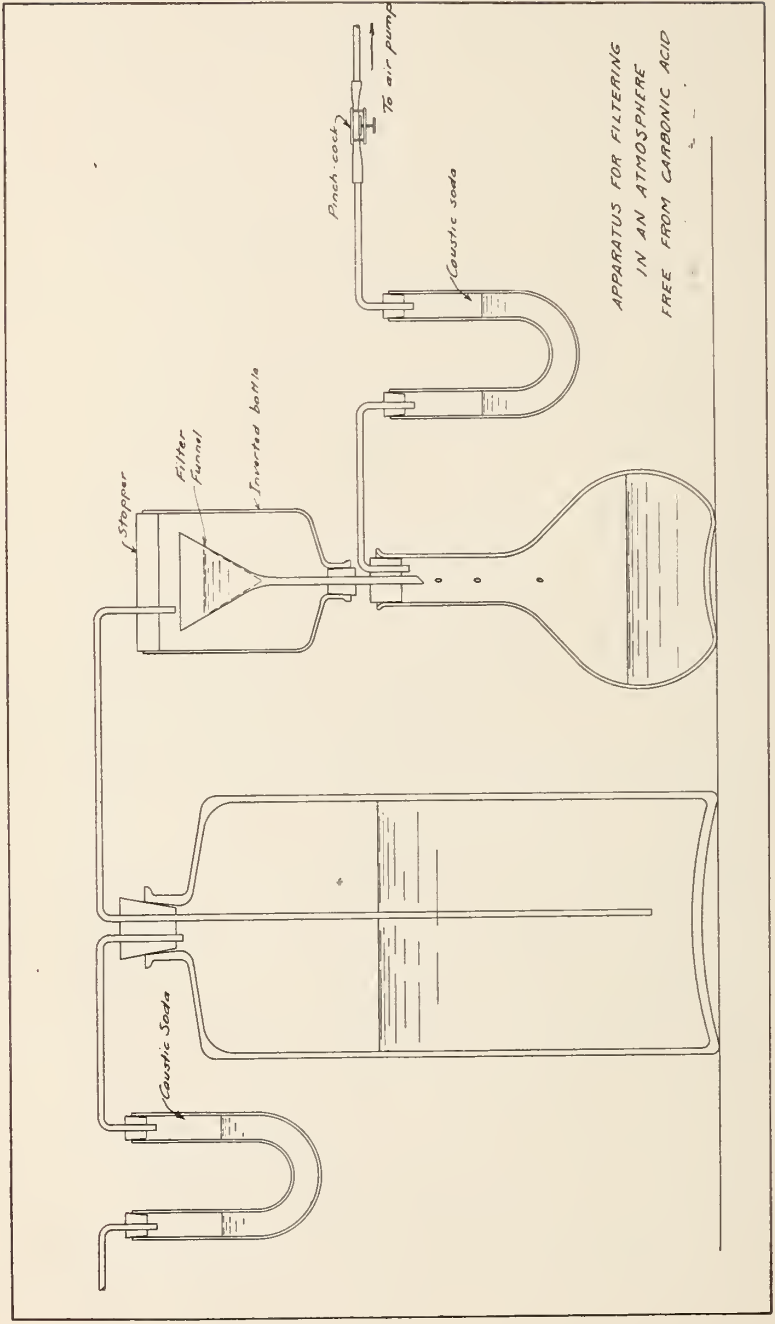
TIME	TEMPERATURE ° C.	ALKALINITY	
		Phenolphthalein	With Methyl Orange
24 hours	37.5	...	15.0
53 "	"	7.0	15.0
77 "	"	6.5	14.5
	FLASK 2.		
28 hours	23	6.5	14.0
53 "	22	6.0	13.0
76 "	20	6.5	13.0

The N/50 H_2SO_4 used in all this work was prepared from normal sulphuric acid, which was checked by titration against pure sodium carbonate, and by the gravimetric determination as barium sulphate. The dilution was done by means of a pipette and flask, which were calibrated against each other.

Another experiment, carried out in practically the same manner as those above described, at 20° C., gave 13 parts per million.

A value of 13 was also obtained by an experiment in which pure water, free from CO_2, was boiled, with an excess of chemically pure $CaCO_3$, for three hours in a flask of Jena glass, and allowed to stand with frequent agitation, protected from the CO_2 of the air as above described, for a period of four days.

The alkalinity of the solution of $CaCO_3$, using phenolphthalein as indicator, should be equal to one-half of the value obtained by using methyl orange as indicator. It was found, however, when the solution was filtered in the air, that enough CO_2 was dissolved to reduce the phenolphthalein figure. In order to overcome this difficulty, an apparatus was made to filter solutions out of contact with CO_2. (See diagram.) This apparatus, although accomplishing its object, consumed considerable time, as it was necessary, before attempting filtration, to remove from the apparatus every trace of CO_2 gas by aspiration. The method finally adopted for securing the $CaCO_3$ solution free from suspended particles, as well as CO_2, consisted in allowing all the $CaCO_3$ to settle, after which 100 c.c. of the clear



APPARATUS FOR FILTERING IN AN ATMOSPHERE FREE FROM CARBONIC ACID supernatant solution could be drawn off with a pipette attached to a filter pump by a rubber tube. In every case in which this was done, all error due to the presence of CO_2 in the air was avoided, and the phenolphthalein value was equal to just one-half the methyl orange value, as it should be. (See Table 2.)

In order to throw some light upon the high figures obtained in the preliminary work mentioned, experiments were conducted with flasks of Jena and Bohemian glass, in exactly the same manner as those last described, but omitting the addition of $CaCO_3$. The results of these blank tests are given in the following table:

TABLE 3.

TIME OF BOILING	TIME OF STANDING	ALKALINITY (PARTS PER MILLION)							
		Flask 1		Flask 2		Flask 3		Flask 4	
		P.*	M.†	P.	M.	P.	M.	P.	M.
1 hour......	0 days	..	1.0	..	..	0.5	2.0	..	..
2 hours.....	4 "	..	..	..	..	..	..	10.5	12.5
2 "	3 "	..	..	0.75	1.5	..	..	..	..
3 "	0 "	0	..	..	..	4.0	6.5	..	..
4½ "	0 "	0	1.5	..	..	5.5	9.5	..	..
4¾ "	5 "	0	2.5	..	..	7.0	10.0	..	..

*Phenolphthalein. †Methyl orange.

1 and 2 Jena glass flasks. 3 and 4 Bohemian glass flasks.

As the presence of small amounts of alkali in solution accelerates the action of water upon glass, and as the flasks used in the preliminary experiments were, with one exception, made of Bohemian glass, it can be readily seen why the figures first obtained were so high and variable. If, however, we subtract the average figure obtained in the blank experiments on Bohemian flasks, namely, 11.25, from the values obtained in the preliminary experiments, in which that kind of glass was used, we obtain values of 12 to 19 parts per million.

A better figure, however, is arrived at by subtracting from the results obtained in Jena flasks the corresponding blank, namely, 1.5, which gives us, in round numbers, 12 parts per million at 22° C., and 14 parts per million at 37.5° C.

The figures for the solubility of normal calcium carbonate, i. e., from 12 to 14 parts per million, were corroborated by other experiments described later, in which calcium bicarbonate solution was treated with lime water. The solubility of $CaCO_3$ in water is influenced by the presence of other substances. Salts of ammonia, for example, tend to render it more soluble, while other substances decrease the solubility. We found, for instance, that in water containing 500 parts per million of pure $CaSO_4$, the solubility of $CaCO_3$ was 4.5 parts per million at room temperature, while in water containing 1,000 parts per million $CaSO_4$, the solubility of $CaCO_3$ was 4.0 parts per million. The effect of the presence of various salts on the solubility of $CaCO_3$, on account of its importance, has been left for subsequent discussion in detail.

SOLUBILITY OF MAGNESIUM HYDROXIDE.

When magnesium carbonate is precipitated from aqueous solution with alkaline salts, the product is invariably basic, that is to say, the carbonate contains a certain amount of hydroxide. The exact composition of this precipitate varies according to conditions, temperature being a very important factor. The solubilities of normal magnesium carbonate or basic magnesium carbonate, therefore, cannot be definitely fixed. It is certain, however, that all modifications of basic magnesium carbonate are much more soluble than magnesium hydroxide, and in water softening, sufficient lime water is added to precipitate all the magnesium in the latter form, i. e., as MgO_2H_2.

The values for the solubility of MgO_2H_2 as given by most of the early experimenters, quoted by Comey,[1] are so high as to be obviously worthless. The most probable figures, as shown by Table 4, vary from 9 to 20 parts per million:

TABLE 4.

AUTHORITY	DATE	TEMPERATURE	SOLUBILITY OF MgO_2H_2	
			Parts per Million	Equivalent Alkalinity, i. e., as $CaCO_3$
Fresenius[11]	1847	"Ordinary" and 100° C.	18	31
Bineau[12]	1855	"Ordinary"	10–20	17–34
Kohlrausch and Rose[9]	1893	18° C.	9	16

In determining the solubility of this salt, the same method was employed as for $CaCO_3$. The best grade of MgO, freshly ignited in order to decompose any carbonate present, was used.

The results are shown in the following table:

TABLE 5.

TIME IN HOURS	TEMPERATURE	ALKALINITY IN TERMS OF $CaCO_3$	MAGNESIUM HYDROXIDE (PARTS PER MILLION)	
			Observed	Corrected for Blank
120	22° C.	23	13	12
144	"	23	13	12

Both experiments were conducted in flasks of Jena glass.

This method for MgO_2H_2 presents considerable difficulty. Magnesium hydroxide is a colloidal precipitate, which requires a long time to settle, and tends to pass through filter paper. Therefore the results may be somewhat in excess of the true value.

PRECIPITATION OF LIME AND MAGNESIA WITH LIME WATER.

Although $CaCO_3$ and $MgCO_3$ are with difficulty soluble in pure water, they dissolve readily in water containing CO_2 to the extent of about 1 g. per liter for $CaCO_3$, and 20 g. per liter for $MgCO_3$, at ordinary temperature and pressure. The state in which these salts exist in solution is not definitely established, but they may be represented respectively by the formulas $CaCO_3$, CO_2, H_2O, and $MgCO_3$, Co_2, H_2O. They are precipitated from solution by lime water according to the following equations:

$$CaCO_3,\ CO_2,\ H_2O + CaO_2H_2 = 2CaCO_3 + 2H_2O \quad \text{and}$$
$$MgCO_3,\ CO_2,\ H_2O + 2CaO_2H_2 = MgO_2H_2 + 2CaCO_3 + 2H_2O.$$

In order to corroborate the evidence already obtained as to the solubility of $CaCO_3$ and MgO_2H_2, and also to throw some light upon the course of the reactions which take place when bicarbonates of lime and magnesia are precipitated with lime water, the following experiments were undertaken.

The solutions used were prepared as follows:

Pure $CaCO_3$ was suspended in distilled water, and CO_2 gas passed in for some time. After the undissolved $CaCO_3$ had settled, the supernatant fluid was poured into a large evaporating dish, and allowed to stand over night, thus allowing the excess of free CO_2 to escape. The liquid was then filtered, giving a fairly strong solution of pure calcium bicarbonate, which could be diluted to any strength desired. Magnesium bicarbonate was prepared in a similar manner from the purest obtainable carbonate of magnesium.

Lime water was made in the usual manner, by shaking distilled water with an excess of freshly slaked lime and filtering the resulting solution. For some experiments a chemically pure lime was used, but the solution obtained from this differed in no appreciable respect, for this purpose, from that obtained with good commercial lime. The strength of these solutions was determined by titration with N/50 H_2SO_4, using phenolphthalein as indicator in the case of lime water, and methyl orange in the case of the bicarbonates. The precipitation experiments were performed as follows: The desired amount of bicarbonate solution was measured with as much accuracy as possible into a bottle of flint glass with a rubber stopper, and brought to the desired temperature. The volumes used varied from 1.8 to 3.5 liters, in bottles of 2 and 4 liters capacity, respectively. 100 c.c. was then quickly withdrawn, and the amount of free CO_2 determined by titration with carefully prepared N/22, Na_2CO_3 solution, using phenolphthalein as indicator. Then, the alkalinity and volume of the bicarbonate solution, the amount of free CO_2 gas, and the strength of the lime water being known, a simple calculation sufficed to determine the exact amount of lime water necessary to cause the desired reaction. After the addition of lime water the contents of the bottle were thoroughly mixed by shaking, and maintained at the desired temperature as long as necessary. The amount of calcium carbonate in solution was determined, when desired, by withdrawing a portion of the solution, filtering quikly through paper, and titrating with N/50 H_2SO_4 as usual.

PRECIPITATION OF CALCIUM CARBONATE.

In the experiments where calcium bicarbonate alone was used, the precipitate which first formed upon adding lime water was colloidal in character, but gradually changed, becoming crystalline in about 20 minutes. This change took place more quickly at higher temperatures than at low temperatures. At 38° C. it required only about 10 minutes, while at 9° C. it required nearly 30 minutes.

Three preliminary experiments with calcium bicarbonate, which were conducted at room temperature as described above, gave the results shown in Table 6. Just enough lime water was added to neutralize all the free CO_2 and precipitate all the calcium as carbonate.

TABLE 6.

Experiment Number	Time of Standing in Hours	Temperature	Parts per Million $CaCO_3$
I	1	22° C.	92
II	3.5	22	35
III	6	22	26

It will be noticed that the amount of $CaCO_3$ remaining in solution was inversely proportional to the time of standing, and it is evident, therefore, that the time element is of much importance in this reaction.

In order to investigate this question more thoroughly, another experiment, like those just described, was carried on at room temperature. Portions of the solution were withdrawn for analysis at intervals, until the amount of $CaCO_3$ remaining in solution had reached a minimum.

The results obtained are shown in Table 7.

TABLE 7.

Time in Hours	Temperature	$CaCO_3$ (Parts per Million)
0	....	180*
1	19° C.	70
3	20	60
6	20	43
24	22	22
50	22	18
74	19	17
122	20.5	14.5
218	20.5	15
720	20.5	17.5

*Calcium bicarbonate solution before addition of lime water.

In these, as in most of the experiments, the initial alkalinity of the water made no appreciable difference in the rapidity of the reaction after the solution had stood a few hours. In all cases, the results obtained by using phenolphthalein as indicator were one-half of those obtained with methyl orange; and this was found to be true at all stages of the reaction. The experiment recorded in Table 7 was repeated, and figures were obtained which practically checked those given.

The above experiment was then twice repeated, but in one case the solution was kept at a temperature of 2° C., and in the other case at a temperature of 35° C., the other conditions being the same as above. The results of these experiments are given in Tables 8 and 9.

TABLE 8.

Time in Hours	Temperature	Parts per Million of $CaCO_3$
0	1° C.	170*
1	2	313
4	2	59
6	2	49
24	2	28
52	2	18
77	2	17
173	2	15.5
312	2	15.5

*Before adding lime water.

TABLE 9.

Time in Hours	Temperature	Parts per Million of $CaCO_3$
0	35° C.	197*
0.25	35	43
0.75	35	29
5.75	35	24
28.0	35	18.5
54.0	35	16.0
72.0	35	18.0

*Before adding lime water.

These experiments on the precipitation of calcium carbonate with lime water agreed in giving an end point which was practically constant, even though the temperature varied considerably. Thus, at 2° C. the end point showed the solution to contain 15.5 parts per million of calcium carbonate; at 20° it was 14.5; and at 35° it was 16. In these experiments the alkalinity nearly always increased slightly, after attaining a minimum value, but this was evidently due to the action of the solution on the glass of the bottle. It is evident from Tables 8 and 9 that the speed of the reaction, after the first hour, was greater at 35° than at 2°. It was noticed that in the experiment where the water was kept at 2° the calcium carbonate did not come down as a colloidal precipitate, but gradually separated from the solution in very fine crystals.

PRECIPITATION OF MAGNESIUM HYDROXIDE WITH LIME.

The experiments on the precipitation of magnesium hydroxide with lime were carried on in the same manner as those for calcium carbonate. Two experiments were made at first, one at 20° C. and one at 37° C. The results of these experiments are given in Tables 10 and 11.

TABLE 10.

Time in Hours	Temperature	Observed Alkalinity	Alkalinity Due to MgO_2H_2
0	22° C.	292*	..
0.25	21	202	..
0.75	21	104	29
9.0	20	62	23
27.0	20	58	37
99.0	20	47	21
195.0	20	32	17
267.0	20	33	17

*Before adding lime water.

TABLE 11.

Time in Hours	Temperature	Observed Alkalinity	Alkalinity Due to MgO_2H_2
0	31° C.	220	..
1.5	31	73	45.0
5.0	34	56	32.0
29.0	37	45.5	27.5
53.0	37	34.5	17.5
101.0	37	29.0	15.0
297.0	37	28.0	15.0

In these experiments the observed alkalinities represent, of course, not only the magnesium hydroxide, but also the calcium carbonate derived from the lime water added. In order to obtain figures for the magnesium hydroxide alone, there were subtracted from the observed alkalinities the amounts of normal calcium carbonate present in solution at corresponding times and temperatures, as shown by those experiments in which $CaCO_3$ alone was precipitated. These results are given in the last columns of these two tables.

It will be seen from these experiments that the reaction with lime water proceeds more rapidly in the case of magnesium bicarbonate than in the case of calcium bicarbonate.

In order to obtain better values for MgO_2H_2, two precipitation experiments were made together, under the same conditions of temperature and time, one with calcium bicarbonate, the other with magnesium bicarbonate, the temperature being about 35° C. The results of this experiment are given in Table 12.

TABLE 12.

TIME IN HOURS	ALKALINITY IN PARTS PER MILLION		
	Calcium Carbonate	Magnesium Hydrate and Calcium Carbonate	Difference (MgO_2H_2)
0.25	55	65	30
0.5	40	66	26
1	33	60	27
2	29	55	26
3	28	53	25
5	25	50	25
10	22	48	26
24	18.5	39	20.5
48	16	36	20

The reason why the magnesium reaction takes place more rapidly than the calcium may be due to the fact that in the former case the precipitate is colloidal in character, while in the latter case it is crystalline, although colloidal for a short time at first. It apparently takes some time for calcium carbonate to precipitate completely in this crystalline form.

These facts have an important bearing upon the practical operation of water-softening plants. Since the reaction of lime water on the bicarbonates of calcium and magnesium is not instantaneous, but requires a certain amount of time for completion, it follows that, in order to obtain the best results, water-softening plants must be designed of such capacity as to give time for this reaction to be pretty well advanced before the water leaves the settling tanks. A study of the practical operation of a number of plants has shown that, where the capacities of the coagulation basins are large, the results are better than where they are small. Thus, at Winnipeg, Manitoba, where the time interval is less than two hours, the resulting alkalinity of the water is 80 parts per million, while at Oberlin, Ohio, where the time interval is more than one day, the resulting alkalinity is sometimes as low as 28 parts per million. In certain plants where the time interval is six hours, the results are intermediate between those mentioned.

It is a well-known fact that in most water-softening plants where recarbonization is not practiced, "after-deposits" of lime are apt to form on the sides of the settling tanks, on the sand grains of the filter, and in the distribution pipes. Analyses of these deposits made by the writers have shown them to be composed almost entirely of calcium carbonate, with almost no magnesium. The reason for this appears to be connected with the fact that the rate of the reaction in the case of calcium is slower than in the case of magnesium, and partly, of course, to the fact that the calcium content of most waters considerably exceeds the magnesium content. In one case recently called to our attention, the sand in the filter had become so encrusted with calcium carbonate that its effective size had increased from .40 to .60 mm. These "after-deposits" may be prevented by resorting to the method of recarbonization, which supplies to the water enough free carbonic acid to redissolve, as bicarbonates, the excess of calcium carbonate, and perhaps magnesium hydroxide, which would otherwise gradually settle out.

SUMMARY AND CONCLUSIONS.

1. The solubility of normal calcium carbonate, as determined by direct experiment, was found to vary from 12 to 14 parts per million; by the precipitation experiments with lime water it was found to be about 14.5 parts per million; but this figure is subject to a subtractive correction on account of the action of the alkaline solution on the glass. The most probable value for the solubility of this salt, at ordinary temperatures, may be taken, therefore, as 13 parts per million.

2. The solubility of magnesium hydrate, as determined by direct experiment, was found to be 12 parts per million. By the precipitation experiments with lime it was found to be 10 parts per million, which is probably more nearly correct, and which may be taken as the most probable value. This is equivalent to an alkalinity of 17 parts per million.

3. The reaction between lime water and calcium bicarbonate requires several days for its completion at ordinary temperatures, but is much more rapid at 37° C. than at the freezing point. The greater part of the precipitate, however, separates within six hours at ordinary temperatures. The longer the period which can be allowed for the reaction, the better, therefore, is the result.

4. The reaction between lime water and magnesium bicarbonate takes place somewhat more rapidly than in the case of calcium. This is apparently due to the fact that the precipitate formed is colloidal instead of crystalline.

The delayed reaction in the case of calcium explains why "after-deposits," so often found in water-softening plants, contain so little magnesium.

5. In order to prevent the formation of "after-deposits" some method of recarbonization is necessary when the time allowed for the reactions is short.

6. The effect of the presence of different salts on the solubility of calcium carbonate and magnesium hydroxide was not determined in these experiments, but left for more complete study.

APPENDIX.

1. A. M. COMEY. *A Dictionary of Chemical Solubilities*, 1896.

2. CHEVALET. *Ztschr. f. anal. Chem.*, 1869, 8, p. 91.

200 c.c. of $CaCO_3$ solution were distilled with five grains NH_4Cl. The first 100 c.c. of the distillate were passed into 10 c.c. dilute H_2SO_4. The CO_2 was boiled off and the still uncombined acid determined volumetrically. No details are given as to method of making $CaCO_3$ solution.

3. HOFFMANN. *Ztschr. f. anal. Chem.*, 1865, 4, p. 414.

A solution of calcium bicarbonate was subjected to prolonged boiling and the filtrate analyzed. It is not stated whether the boiling was conducted in glass, porcelain or platinum.

4. PELIGOT, mentioned by Bineau (*vide sq.*) as having obtained a value of 20 parts per million. No reference given.

5. WELTZIEN. *Ann. d. Chem. u. Pharm.*, 1865, 136, p. 165. No particulars of method given.

6. BINEAU. *Ann. de chim. et phys.*, Par., 1857 (3), 51, p. 290.

Solutions of $CaCO_3$ were obtained, (1) by shaking an excess of $CaCO_3$ with pure water, (2) by adding a slight excess of calcium salt to dilute solution of Na_2CO_3, and (3) by prolonged boiling of calcium bicarbonate solution. The amount of $CaCO_3$ in solution was determined by the addition of an excess of dilute H_2SO_4, expelling CO_2 by heat or in a partial vacuum, and titrating back with standardized lime water. Special precautions were taken against the action of the CO_2 of the atmosphere, and the action of $CaCO_3$ solution on glass. "The result in which the author places most confidence is 16 parts per million and the solubility of $CaCO_3$ is certainly not more than 20 parts per million." These experiments were evidently made with great care.

7. SCHLÖSING. *Compt. rend. Acad. d. Sc.*, Par., 1872, 74, 1552.

"The solubility of normal $CaCO_3$ in pure water was determined, with all necessary precautions, at 16° C."

8. HOLLEMAN. *Ztschr. f. physik. Chem.*, 1893, 12, 125.

Carefully prepared $CaCO_3$ was agitated with water of great purity in small vessels, protected from the CO_2 of the air by tubes of soda lime. The electrical conductivity of the solution thus obtained was carefully measured, and the amount of salt in solution calculated from this figure. The method was first checked by solutions of known composition, and found to be accurate within about 1 per cent, the greatest deviation being 2.8 per cent.

9. KOHLRAUSCH AND ROSE. *Ztschr. f. physik. Chem.*, 1893, 12, p. 241.

Pure $CaCO_3$ was added to water of great purity, in flasks, well shaken, and the electrical conductivity determined until it reached a constant value, from which the solubility of $CaCO_3$ could be calculated. The solution was protected from the CO_2 of the air and special attention was paid to the quality of the glass used. An exactly similar method was used to determine the solubility of MgO_2H_2.

. . . . Kohlrausch and Rose adduce the following advantages for the electrical conductivity method of determining solubilities, which they claim is the best method for this purpose, when the values to be determined are small:

The progress of solution can be followed, and when a constant value is attained, the fact is immediately recognized.

There is no necessity for separating from the solution the excess of undissolved substance, since the latter introduces no perceptible error in the determination. Hence, filtration as well as evaporation and other lengthy operations, which involve the possible introduction of impurities, are avoided, and the substance can be used in as finely divided condition as may be desired.

Only a small amount of substance is required; the determination requires but a short time, thus avoiding errors introduced by the action of the solvent upon the containing vessel.

The determination is conducted in closed vessels, thus avoiding the influence of foreign matter, for instance, the CO_2 of the air.

10. KIMBERLY. *Jour. Infect. Dis.*, 1905, Supplm. No. 1., p. 157.

11. FRESENIUS. *Ann. d. Chem. u. Pharm*, 1847, 59, p. 117.

Carefully purified MgO was digested with pure cold water, filtered, and the filtrate evaporated to dryness in platinum dish. No mention is made of precautions taken against action of CO_2 of the air or the action of MgO_2H_2 solution on glass.

12. BINEAU. *Compt. rend. Acad. d. Sc.*, Par., 1855, 41, 510.

Method not given. Author states that solubility is much increased by action of CO_2 of air.

EXPERIENCE WITH THE USE OF A NONBASIC ALUM IN CONNECTION WITH MECHANICAL FILTRATION.

GEORGE C. WHIPPLE AND FRANCIS F. LONGLEY.

THE chemical which is used most commonly for the coagulation of waters, in connection with the mechanical system of filtration, is basic sulphate of alumina, more often referred to as "sulphate of alumina," or merely as "alum." In a case where so much depends upon the proper chemical treatment of the water, it is evident that the quality of the chemical used is a matter of fundamental importance. Most of the products which have been furnished for this purpose have been reasonably satisfactory in this respect. Recently, however, an instance has occurred where inferiority in the quality of the alum has caused a noticeable diminution in the efficiency of the filter. The writers have thought that a brief description of this experience might be of interest to others.

Theoretical sulphate of alumina has the formula $Al_2(SO_4)_3 . 18\ H_2O$; that is, it contains 48.64 per cent of water, 15.32 per cent of alumina (Al_2O_3), and 36.04 per cent of sulphuric acid (SO_3). The aluminum sulphate of commerce, however, differs somewhat from these theoretical proportions. It usually contains somewhat less water of crystallization and more sulphuric acid and alumina. The excess of alumina over the theoretical amount is generally greater than that of the acid, in the case of alums which have been furnished for filtration purposes. This is what has given rise to the trade name "basic sulphate of alumina." The excess of alumina (Al_2O_3) over the theoretical amount necessary to combine with the acid (SO_3) usually varies from 1 to 5 per cent, although it sometimes is as high as 10 per cent. The ratio of alumina (Al_2O_3) actually present in an alum to the amount theoretically necessary to combine with the acid (SO_3) present has been sometimes termed the ratio of basicity. While in the case of most alums which have been furnished for filtration purposes the basicity ratio has exceeded unity, alums are sometimes manufactured for other purposes which have a basicity ratio less than unity or which are exactly neutral. Neutral or acid alums are sometimes preferred in connection with paper-making, bleaching, etc. It was the use of a neutral alum in connection with mechanical filtration that gave rise to the investigations here recorded.

The sulphate of alumina of commerce is seldom absolutely pure. It usually contains a certain amount of matter which is insoluble in water, small amounts of ferrous and ferric iron, and, occasionally, traces of lime, etc.

Specifications under which alum is obtained for filter purposes usually require that the amount of insoluble matter, the amount of iron, etc., shall not exceed certain fixed quantities. They also provide that the amount of available alumina (Al_2O_3) shall not be less than a certain specified figure. Seldom or never, however, has it been specified that there shall be an excess of basic alumina, that is, that the alum shall contain more alumina than is theoretically necessary to combine with the sulphuric acid present. Our experience, however, has led us to believe that this is an important point.

A few months ago a mechanical filter was put into operation, and from the start it gave lower bacterial efficiencies than it should have done, considering the character of the plant. The percentage removal of bacteria was between 90 and 95 per cent, instead of 98 per cent, as was expected. This condition of affairs went on for some time, during which the plant was examined in every part, and everything found to be working satisfactorily and in accordance with the most improved regulation. The character of the sand, the method of washing, the rate of wash water, the application of air, the time of coagulation, etc., were all found to be as they should be, yet the results remained unsatisfactory. After a somewhat long series of experiments it was finally determined, by process of exclusion, that the trouble must lie with the alum. In the meantime it had already been noticed that the alum furnished had a low basicity ratio or was practically neutral. An occasional lot was even found to be slightly acid instead of basic. There being no experience on record with the use of such an alum, laboratory experiments were made to determine the difference between a neutral alum and a thoroughly basic alum in the coagulation of water. Several samples were obtained which differed in their basicity from 1.00 to 1.06, and these alums were added to various samples of water in various amounts from one-half to three grains per gallon. The results were striking. In the case of the basic alums the flocs of coagulant formed more rapidly and were much larger than in the case of the neutral alums. This was found to be true with a clear hard water, with a soft water moderately clear, and with a highly colored water. The neutral alum was then rendered basic by the addition of sodium hydrate, and when this was compared with the same alum without this addition, the flocs were decidedly larger in the case of the basicified sample. The reason for this action is not apparent, but the fact appears to be beyond dispute. It was interesting to note that the application of sodium carbonate to the water before adding the alum did not have the same effect as the addition of sodium hydrate to the alum itself. These experiments were then repeated on a large scale in the actual operation of the filter. Caustic soda was purchased and added to the alum solution in the tanks, but this did not appear to give any improvement in the results of filtration, while it did give rise to a noticeable sludge in the bottom of the alum tanks. It was evident that this method of application was not the proper one, for, as shown by laboratory experiment, although the sodium hydrate dissolved with reasonable care, especially on being warmed, it precipitated within a short time, and the solution apparently lost its basic property. Accordingly the soda solution was made up separately from the alum, and an arrangement was made whereby it was applied to the alum solution just before the latter was added to the water. This method of application appeared to accomplish its purpose, and there was an immediate improvement in the efficiency of the plant, as shown by the following figures; the first line represents the period during which the neutral alum was used without soda; the second with soda:

TABLE 1.

BACTERIA PER C.C.			COLOR		POUNDS PER MILLION GALLONS	
Raw Water	Filtered Water	Per Cent Reduction	Raw Water	Filtered Water	Quantity of Alum	Quantity of Soda
4,370	391	91.0	45	13	267	0
2,520	122	95.2	45	6	237	5

Not only were the bacterial efficiency and the color of the effluent improved, but it was found that equally as good results could be obtained by the use of less alum when the soda was used as when soda was not used. These experiments were so convincing that it was decided to purchase a new lot of alum which had a basicity ratio of at least 1.05. The results obtained with this new lot of alum showed a great improvement over previous ones, a fact made evident by the figures given below. In order to make a further test of the advantages of the basic alum, the use of the two alums was alternated from time to time in order to obtain comparative results.

TABLE 2.

Dates	Average No. Bacteria per c.c.			Quantity Alum*	Alum
	Raw Water	Filtered Water	Per Cent Reduction		
Mar. 27–30	10,130	500	95.4	199	Neutral alum
Mar. 31–Apr. 4	19,755	78	99.4	303	Basic “
April 5–6	13,400	152	98.7	294	Neutral “
April 7–8	3,810	73	98.2	265	Basic “
April 9–10	1,390	74	95.0	278	Neutral “
April 11–29	2,212	77	96.7	223	Basic “
Apr. 30–May 8	2,314	135	94.5	223	Neutral “
May 9–18	996	43	95.8	228	Basic “
May 19–31	1,423	99	93.0	260	Neutral “
Average	4,022	176	95.5	244	Neutral alum for 30 days
Average	4,374	68	98.4	238	Basic alum for 40 days

*Pounds per million gallons.

The figures show that with the neutral alum the bacterial efficiency for 30 days was 95.5 per cent, while with the basic alum for 40 days it was 98.4, even though the quantity of basic alum used was slightly less than in the case of the neutral alum. Since that time the use of basic alum has been insisted upon, and the efficiency of the filters has been satisfactory.

The following table gives the results of chemical analyses of typical lots of neutral and basic alums which have been used:

TABLE 3.

	Lot No. 3	Lot No. 5	Lot No. 7	Lot No. 9	Lot No. 10	Lot No. 15
Available Al_2O_3	17.24	17.23	17.18	17.56	17.95	17.57
Total SO_3	40.92	40.70	41.43	40.35	40.17	40.05
Free SO_3	0.20	0.18	0.27	0.23	0.14	0.15
FeO	0.05	0.042	0.05	0.05	0.17	0.20
Fe_2O_3	0.27	0.28	0.27	0.23	0.14	0.01
Insoluble matter	0.09	0.26	0.14	0.06	0.08	0.25
Basicity ratio	0.995	1.00	0.982	1.025	1.05	1.03

The neutral alums were found to be unsatisfactory in another respect. When dissolved in the alum tanks the solution was turbid, and when the tank was emptied a considerable amount of sludge was found at the bottom. This insoluble matter caused no little trouble with the small centrifugal pumps which were used in pumping the solution to the tank where its rate of application to the water was controlled. The suspended matter in the alum solution formed deposits in the pipes, which had to be frequently cleaned out in order to maintain a continuously uniform flow. It also necessitated frequent cleaning of the alum tanks. Some of the sediment was due to the presence of ferric iron, but a part of it appeared to be due to undecomposed bauxite used for the manufacture of the alum. The basic alums which have been used have not given nearly as much trouble in this regard as the neutral alums.

The sludge that formed in the bottom of the tank was detrimental, however, only in a mechanical way, that is, by preventing the uniform application of the solution to the water. It did not affect the active strength of the solution (i. e., the available Al_2O_3), as the following figures will show. Samples of the alum solution were taken from the tanks and allowed to stand in bottles for 24 hours. Then equal portions were taken, of A the clear solution at the surface; and B the solution containing the sludge at the bottom. Gravimetric determinations of the alumina in these samples gave results as follows:

TABLE 4.

	A	B
Sample No. 1	0.0078 grams A1203	0.0867 grams A1203
" " 2	0.0770 " "	0.0777 " "
" " 3	0.0899 " "	0.0910 " "

Alums furnished by different dealers vary considerably with respect to their iron contents. In some cases the iron is present chiefly in the ferrous condition, and in other cases in the ferric. It has been the habit of some filtration engineers to specify that the iron shall be present chiefly in the latter form, fearing that in the ferrous condition it would be more likely to unite with the organic matter, pass through the filter, and remain in the filtered water. Our experience has been that this fear is unfounded, and that there is an advantage in having the iron present in the ferrous form instead of in the ferric. The resulting solution appears to be much clearer, and there is less sludge formed in the tank, less clogging of the pipes, and less interference with the regulation of the alum feed. There is, however, a limit beyond which the amount of iron should not be allowed to pass. Laboratory experiments which we conducted in connection with the matter, as well as experiments made with the entire plant on a larger scale, showed that an amount of ferrous iron equal to 0.5 per cent of the alum gave no trouble. It increased neither the color of the filtered water nor the amount of iron which it contained.

As a result of our experience the following specifications have been drawn up for the purchase of future supplies of alum, and we suggest that specifications for filter alum shall always require that there shall be a substantial excess of alumina, that is, that the alum shall be distinctly basic. This is usually so in any case when the available alumina exceeds 17.5 per cent.

SPECIFICATIONS.

The basic sulphate of alumina shall be guaranteed to contain 17 per cent of alumina (Al_2O_3), soluble in water, and of this amount at least 5 per cent shall be in excess of the amount theoretically necessary to combine with the sulphuric acid present. It shall not contain more than 0.1 per cent of matter insoluble in water and it shall be practically free of chips and debris of all kinds. It shall not contain more than 0.5 per cent of iron (Fe_2O_3), and the iron shall be preferably in the ferrous state. The alum shall be crushed to pieces of such a size that they will pass through a ring three inches in diameter.

If bidders so desire, they may bid on a product containing a larger amount of soluble alumina, stating, however, the amount which they guarantee. The additional strength will be taken into account in deciding where to place the order

THE USE OF COPPER SULPHATE IN WATER FILTRATION.

H. W. Clark and S. DeM. Gage.

An experiment was begun at the Lawrence Experiment Station, during 1904, in order to test the value of copper sulphate as an aid in the purification of polluted water. In this investigation a a large experimental filter, 17 feet 4 inches in diameter and containing two and one-half feet in depth of good filter sand, was used. Before the beginning of the copper sulphate experiment, this filter had been in use for 11 years, filtering Merrimac River water. Beginning May 17, 1904, however, copper sulphate was added to the raw water applied to this filter, the amount used at first being one part of the sulphate in 1,000,000 parts of water, and the greatest amount used during the year of experiment being one part in 133,000 parts of water.

The copper sulphate was applied directly to the water upon the surface of the filter, but the volume of water always above the sand allowed a storage of water, after introduction of the sulphate, ranging from five and one-quarter hours to slightly more than eight hours, varying, of course, with the rate of filtration at which the filter was being worked.

Examination of the sand of the filter from time to time, during the experiment and after the experiment had ended, showed the accumulation of a large amonnt of copper in the sand, and also showed that the copper penetrated throughout the entire depth of the filter. At the end of the experiment, the amount of copper in the surface sand of the filter was 22.8 parts per 100,000; at a depth of 12 inches, 7.6 parts per 100,000; and at a depth of 24 inches, 6.8 parts per 100,000. Giving these results in terms of copper sulphate, the surface sand contained 89.5 parts of copper sulphate per 100,000; at a depth of 12 inches, 79.8 parts; and at a depth of 24 inches, 26.6 parts.

Anlayses of the effluent of the filter showed that this effluent contained copper, calculated as copper sulphate, varying in amounts at different times from 1.2 parts per million parts of water to 5.8 parts per million parts of water. At the end of August, 1905, three months after we had ceased to add copper sulphate to the applied water, the effluent contained copper, calculated as copper sulphate, to the amount of 3.7 parts per million parts of water, showing that copper was continually being taken into solution from the deposit within the filter.

The actual volume of water passed through this filter daily varied from 14,000 to 26,000 gallons, and the rate of filtration varied from 2,800,000 gallons per acre daily to 5,200,000 per acre daily.

In connection with this work, experiments were made in regard to the rate of sedimentation of copper sulphate after being mixed with water in large tanks at the experiment station, using, of course, water of the same character as that used in the filtration experiment. In one of these sedimentation experiments it was found that there was practically no sedimentation of the copper until after a period of 20 days; in one, a sedimentation of 50 per cent in 54 days; in one, a sedimentation of about 60 per cent in 62 days; and in one, a sedimentation of 58 per cent in 21 days. No sedimentation occurred in periods of 24 or 48 hours.

If, therefore, in this filtration experiment, a sedimentation tank had been placed between the point of application of the copper sulphate and the filter, a normal period of sedimentation would have effected little copper removal, and practically all the copper not passing through the filter and appearing in the effluent would have collected upon the filtering material.

During the active period of this investigation, samples of the water passing to the filter and the effluent of the filter were taken daily for bacterial examination. The bacterial results obtained from the filter during this experiment, when compared with those obtained during the year previous to the application of copper sulphate to the raw water, show no gain in bacterial removal on account of the use of the copper sulphate—rather the reverse. During the year previous to the use of copper sulphate, the raw water contained 8,300 bacteria per c.c. and the effluent, 73 bacteria per c.c.—or a bacterial efficiency of 99.12 per cent. During the year of copper sulphate treatment, the raw water contained 7.400 bacteria per c.c., and the effluent of the filter, 114 per c.c —a bacterial efficiency of 98.5 per cent, 0.62 of 1 per cent less than during the previous year. During both years practically every cubic centimeter sample of the raw water that was tested contained B. coli. The effluent of the filter during the year before the copper treatment contained B. coli in 13.5 per cent of the cubic centimeter samples examined, and during the year of copper treatment it was found in 26 per cent of the cubic centimeter samples examined.

Summarizing, it can be said that poorer results in water filtration were obtained when using copper sulphate than when operating the same filter without the use of the copper sulphate; a poorer effluent, organically, was obtained, and there was an accumulation of copper upon the sand in the filter that would eventually—if copper, in the form in which it remains upon the sand, has any strength at all as a bactericide—reduce the efficiency of the filter very greatly; that is, the biological actions upon which good results with slow sand filters depend would be badly impaired.

ON THE BACTERICIDAL ACTION OF COPPER.

H. W. Clark and Stephen DeM. Gage.

Early in 1904, Moore and Kellerman[1] published the results of their studies of the effect of copper salts on the growth of algæ and bacteria, claiming that minute quantities of copper were sufficient to prevent the development of many troublesome algæ in water supplies, and that a large percentage of the bacteria, including all of the pathogenic bacteria, were destroyed as the result of such treatment. In addition, these observers claimed that storage of a few hours in a copper vessel would effectually free any water from pathogenic bacteria.

Appreciating that a method, apparently so simple, would soon be brought into practical application, and realizing that many factors concerning its safety and efficiency yet remained to be worked out, experiments were begun by the Massachusetts State Board of Health to investigate thoroughly all phases of the subject as applied to the treatment of Massachusetts waters. In the course of this work, much information has been acquired as to the action of copper on the bacterial contents of waters of different kinds, and it is with this phase of the problem that the present paper treats.

The experimental data fall naturally under two heads: (1) experiments in which waters were treated with definite amounts of copper as copper sulphate; and (2) experiments in which the water was placed in contact with metallic copper and allowed to absorb an unknown amount of copper. Each of these two lines of investigation may be subdivided as follows:

a) Effect on the total numbers of bacteria in the water.

b) Effect on the numbers of B. coli naturally present in polluted waters.

c) Effect on the numbers of B. coli added to water in the form of laboratory cultures.

d) Effect on the numbers of B. typhosus added to water in the form of laboratory cultures.

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[1] *U. S. Dept. Agri. Bureau Plant Industry, Bull. No. 64*, 1904.

In addition, experiments have been made to compare the action of other salts frequently used in water purification, such as ferrous sulphate and aluminum sulphate, with copper sulphate, and also to compare the effect of other metals with that of metallic copper.

To the sanitarian the question arises: Does copper in dilute solution destroy the bacteria completely, and do the same laws which apply to the common water bacteria apply equally when dealing with the bacillus of typhoid fever? As we have frequently pointed out, tests made with laboratory cultures of B. typhosus, while they have a certain value, are not conclusive evidence as to what would be the behavior of typhoid organisms which have come from fecal matter and have become accustomed to life in water.

The difficulty of isolating typhoid from mixed cultures in water, and the preponderance of other germs in sewage-polluted water, rendered it necessary to attack our problem in a roundabout manner. The method which has given the most reliable information at Lawrence, in investigations of this character, has been to study the relative viability of different laboratory cultures of both B. typhosus and B. coli, to study the viability of the colon bacillus in a natural state under similar conditions, and from the two sets of experiments to draw inferences as to the viability of the typhoid bacillus in nature under similar conditions.

The weak point in the conclusions of Moore and Kellerman with regard to the destruction of typhoid by copper is that they were drawn from analyses in which the largest amount of water tested was 1 c.c., and the usual amount tested was less than .01 c.c. It is generally conceded, especially when dealing with laboratory cultures, that the great majority of the typhoid bacilli are quickly destroyed by conditions unfavorable to their growth. It has also been repeatedly shown that a few germs are much more resistant than the majority, and may survive even under the most unfavorable conditions for many days. All epidemiological evidence points to the conclusion that the germs which are able to live under unfavorable conditions are also extremely pathogenic, and that, while it may help to destroy the majority of the bacilli, no method of sterilizing water is thoroughly effective unless it will accomplish the destruction of the especially resistant individuals.

It is unsafe to conclude that because a certain species of bacteria, especially a pathogen like B. typhosus, is not found in a loopful of the water, or even in 1 c.c., that there is no danger from the use of that water. The average drinking-glass holds about 300 c.c., and until repeated tests of volumes as large as 100 c.c. have been made and the germ proved to be absent, the water under observation cannot safely be said to be free from the test forms.

EXPERIMENTAL METHODS AND EXPRESSION OF RESULTS.

In experiments where the water was treated with metallic salts, the water was first drawn in bulk, carefully mixed and sampled, then divided into portions of uniform size, one of which, the control, was carried through untreated, the others being treated with varying amounts of copper sulphate. The containers in all cases were of glass, and the samples were in every case kept in the dark at room temperature. Daily bacterial analyses were made of the contents of the various bottles in each experiment, and in a few experiments a number of analyses were made during the first 24 hours. In every case the contents of the bottles were shaken thoroughly before samples were removed for analysis, thus insuring a fair sample. The volume of water used in the different experiments varied considerably; in some experiments only 100 c.c. of water were placed in each bottle, in others 1,000 c.c. were used, and in a few experiments 3,000 c.c. was the volume treated.

In the experiments in which the waters have been exposed to metallic copper, about 15 liters of water were used in every case, except in the experiments in which a number of metals were under comparison, in which case the volume of water used was about 1,000 c.c.

The containers in the metal experiments have in some cases been of copper, with the control in enameled ware or in glazed stoneware, and in others the waters have been placed in glass and the metals inserted as thin sheets.

In the experiments where the metal was in the form of the container, or was inserted as sheets, it was impossible to shake the contents, and stirring was inadvisable, since it was likely to cause abrasions in the metal and affect the normal rate of solution of the metal by the water. In these experiments we have resorted to such mixing as could be produced by blowing strongly through the pipettes at the time of sampling. Samples collected under such circumstances are not as representative as would have been the case had a thorough shaking occurred, and some of the variations in such experiments may be attributed to this imperfect sampling.

The determination of the numbers of bacteria and of B. coli in raw waters has been made by the regular "Lawrence" methods. In dealing with sterile waters seeded with laboratory cultures of B. coli and B. typhosus, counts were made on agar plates incubated 18 hours at body temperature, and tests of larger volumes than 1 c.c. were made by mixing broth with the water, incubating at body temperature, and, in case a growth was obtained, by identifying the test organisms by the usual cultural tests. In the following tables the sign + indicates that there was no growth on plates, but that the organism was proved to be present by qualitative tests.

A number of different methods of expressing the copper content of waters have been used in recent publications, the most common being the expression of the ratio of copper sulphate to water by weight. While this method of expression is satisfactory in speaking of treatment with copper sulphate, it is rather out of place in speaking of the amount of copper absorbed in the metallic or colloidal state. As copper sulphate is dissociated in dilute solutions, and as it is the copper *ion* which is apparently the germicide, it is more convenient to express our results in parts of copper per 100,000 parts of water, especially when we wish to compare copper sulphate treatment with the metallic copper treatment. This method of expression has been used throughout in the tables, although in some cases both methods of expression have been used in the text. The following equivalent weights of the metals and of the metallic salts are given for comparison:

Copper sulphate ($CuSo_{4}5H_{2}O$): Copper (Cu)=1:0.253
Ferrous sulphate ($FeSO_{4}7H_{2}O$): Iron (Fe)=1:0.201
Aluminum sulphate ($Al_{2}(SO_{4})_{5}$): Aluminum (Al)=1:0.158

EXPERIMENTS WITH COPPER SULPHATE.

In the following digest of experiments the various bottles in each experiment are designated by the amount of copper added:

Experiment 159.—Merrimac River water. Copper range 0.000253 to 253. Duration 10 days. The bacteria in 0.000253 and 0.00253 act like the control. In 0.0253 and 0.253 the bacteria increased. In 2.53 and 25.3 all but a few of the bacteria were killed, these few remaining throughout the experiment. In 253 the bacteria were all destroyed. The bacterial results are shown in Table 1.

TABLE 1.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	3,800	......	......	......	......	..	..	..
1 day	....	10,000	22,400	340,800	30	9	3	0
2 days	....	4,800	16,000	357,000	88,600	9	0	0
3 "	....	2,500	11,800	300,000	103,000	10	6	1
4 "	....	3,600	9,700	315,000	157,500	12	2	0
6 "	....	1,100	2,400	10,300	79,200	27	0	0
8 "	....	1,800	3,500	7,500	97,500	11	2	0
10 "	....	410	870	4,700	118,800	9	8	0

Experiment 163.—Merrimac River water containing 1 per cent of sewage Range 0.000253 to 253. Duration 20 days. The numbers of bacteria in the control were higher in 24 hours than they were at the start, but decreased slowly during the experiment. The bacteria in 0.000253, 0.00253, and 0.253 were nearly all destroyed in 24 hours, but the few remaining increased to large numbers in the course of three or four days. In 0.0253 the bacteria increased immediately and then decreased slowly. In 2.53 and 25.3 nearly all of the bacteria were destroyed, but the few remaining were alive during 20 days. In 253 all of the bacteria were destroyed in two days. The bacterial results are shown in Table 2.

TABLE 2.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 1 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	46 000	......	......	......	......	..	..	..
1 day	69,000	37	8	333,000	80	20	8	3
2 days	41,500	19	13	404,500	4,200	6	9	0
3 "	12,500	65	34	610,000	3,900	26	23	0
4 "	13,700	447,300	125,700	138,500	4,400	28	35	1
6 "	7,400	590,000	55	83,100	130,800	14	3	0
8 "	8,000	71,000	18,500	220,000	85,200	20	16	0
10 "	4,200	37,350	21	56,200	55,500	15	8	0
13 "	4,400	25,600	23	33,000	15,600	14	12	0
20 "	1,400	100	15	1,700	3,300	7	8	1

Experiment 210.—Merrimac River water containing $\frac{1}{10}$ of 1 per cent of sewage. Duration 131 days. Range 0.000253 to 2.53. The bacteria in the control increased at first and then decreased slowly until the 116th day, when a slight secondary increase was noted. 0.000253, 0.00253, and 0.0253 all increased largely during the first two or

TABLE 3.

Merrimac River Water to Which Was Added 0.1 per Cent of Lawrence Sewage.

(Bacteria per c.c.)

Elapsed Time	Copper—Parts per 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start	17,000	......	......	......	......	....
1 day	72,500	18,500	115,000	132,000	800	150
2 days	64,800	68,400	87,800	87,800	7,000	18
4 “	35,000	5,500	24,300	175,000	372,800	65
5 “	10,700	12,300	32,600	223,200	458,900	65
6 “	17,500	56,500	57,500	162,000	581,400	100
7 “	12,000	15,200	50,500	90,000	151,200	100
8 “	2,200	4,800	7,300	19,500	75,600	50
11 “	11,000	13,300	7,300	35,500	266,300	24
13 “	8,900	4,700	4,600	14,000	532,500	25
15 “	8,000	7,400	3,000	7,500	450,000	2,600
18 “	6,200	5,800	5,100	30,000	770,000	2,400
21 “	3,300	2,400	2,650	9,100	320,000	550
25 “	600	800	900	5,500	18,400	33
32 “	390	450	1,600	4,500	194,400	120
39 “	190	135	180	1,700	210,000	30
46 “	110	210	205	830	11,400	475
53 “	95	475	250	700	10,500	240
60 “	65	90	65	300	8,850	75
67 “	75	90	160	140	4,500	9
74 “	170	85	210	100	1,425	4
81 “	21	275	275	24	480	4
88 “	140	1,100	725	190	175	35
95 “	120	2,700	1,100	530	500	9
103 “	38	75	210	55	230	1
109 “	55	160	350	300	275	5
116 “	1,100	120	5,000	150	325	7
123 “	1,100	750	2,350	90	180	27
131 “	500	400	1,100	400	550	1

TABLE 4.

Merrimac River Water to Which Was Added 0.1 per Cent of Lawrence Sewage.

(B. coli per c.c.)

Elapsed Time	Copper—Parts per 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start	70	..	..	..	..	..
1 day	31	45	32	28	17	10
2 days	2	3	2	0	0	0
4 “	20	7	10	101	13	23
5 “	15	2	17	6	0	0
6 “	43	56	48	47	34	42
7 “	12	11	0	9	2	0
8 “	7	7	8	0	0	50
11 “	12	30	5	3	18	0
13 “	+	3	0	2	4	3
15 “	10	+	0	30	7	6
18 “	0	17	27	41	10	16
21 “	0	10	11	5	13	13
25 “	8	18	25	26	8	11
32 “	0	12	16	3	16	6
39 “	3	0	10	4	0	1
46 “	3	0	3	14	20	14
53 “	0	1	0	5	2	1
60 “	4	1	0	5	0	8
67 “	0	0	7	0	5	3
74 “	2	1	0	0	0	1
81 “	0	0	0	0	0	0
88 “	4	3	2	0	18	5
95 “	1	0	0	0	0	1
103 “	0	0	0	0	0	1

three days and then decreased slowly. In 0.000253 and 0.00253 a secondary increase in the numbers of bacteria was noted on the 88th and 95th days respectively, but no secondary increase of any importance occurred in 0.0253. In 0.0253 the numbers were considerably reduced in 24 hours, but immediately increased to large numbers on the fifth day, and then slowly declined. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and these numbers remained small and gradually decreased throughout the experiment, with the exception of one small secondary increase which occurred from the 15th to the 18th day.

The B. coli in all of the bottles fluctuated considerably, but decreased gradually throughout the experiment, and were found in small numbers up to the 95th day and 103d day, with the exception of 0.253, from which they disappeared on the 67th day. Tests in volumes larger than 1 c.c. were not made. The bacterial results are shown in Table 3, and the B. coli results in Table 4.

Experiment 193.—Tap water containing 3 per cent of sewage. Duration 187 days. Range 0.0000253 to 25.3. The bacteria in the control increased until the second day and then slowly decreased during 96 days, when a large secondary increase occurred, lasting through the 145th day, when a decrease again started. In 0.0000253, 0.000253, and 0.00253 the bacteria all follow much the same laws as the

TABLE 5.

LAWRENCE CITY WATER TO WHICH WAS ADDED 3 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.0000253	0.000253	0.00253	0.0253	0.253	2.53	25.3
Start....	96,400	....	....	....	....	....	....	....
½ hour	93,000	76,400	92,600	44,400	51,000	4,800	24,200	4,600
1 “	76,000	123,000	92,600	73,200	50,000	2,800	7,700	3,100
2 hours..........	106,500	84,200	110,800	60,200	31,900	1,420	6,200	530
4 “	92,300	84,000	132,100	73,000	13,200	350	730	130
6 “	84,400	95,000	140,600	84,400	11,000	210	300	80
8 “	86,400	75,600	114,500	72,500	8,800	150	175	70
24 “	1,000,000	1,120,000	1,150,000	1,150,000	1,500,000	20	2	1
2 days..........	1,100,000	1,400,000	795,200	284,000	1,930,000	220,000	2	0
3 “	108,000	370,000	6,400	324,000	1,420,000	990,000	2	3
5 “	13,400	44,700	32,200	22,600	1,020,000	1,500,000	5	0
7 “	7,500	11,200	16,500	270,000	475,200	276,500	43	5
13 “	2,300	4,300	1,000	4,600	4,100	2,360,000	2,400	4
16 “	2,400	5,500	5,800	5,400	19,500	73,400	6,600	5
21 “	800	4,600	4,800	4,000	11,500	33,800	13,200	1
26 “	3,100	4,800	7,200	6,200	12,800	47,000	32,000	5
33 “	800	6,500	2,000	3,300	8,500	26,500	15	6
40 “	500	6,000	1,250	1,700	5,350	15,500	6	0
47 “	3,400	7,700	4,600	2,500	7,600	11,800	1,080	4
55 “	480	3,700	2,600	1,200	2,400	162,000	6.800	3
61 “	230	185	200	370	440	210,000	4	7
68 “	190	650	390	110	850	....	4	4
75 “	225	325	510	160	1,850	150,000	3	3
82 “	300	310	550	350	600	88,600	2	0
89 “	750	4,700	500	225	275	....	0	0
96 “	300	2,200	250	1,150	510	6,500	0	0
103 “	29,100	20,000	740	1,700	660	41,000	0	0
110 “	75,000	3,200	1,500	650	270	37,500	0	0
117 “	60,000	3,800	475	3,400	300	40,000	0	0
124 “	28,600	10,400	590	1,200	240	25,000	0	0
131 “	22,600	1,300	880	600	300	7,900	0	0
145 “	594,000	5,600	660	725	650	33,500	0	0
160 “	650	1,800	1,100	650	57	8,000	0	0
173 “	6,600	30,900	3,500	5,300	2,000	47,200	0	0
187 “	1,600	2,750	700	300	70	5,900	0	0

control both as to increase, gradual decrease, and a secondary increase after about 100 days. In 0.0253 a primary increase and decrease of bacteria followed the normal curve, only a slight secondary increase being noted. In 0.253 over 99 per cent of the bacteria had disappeared in 24 hours, but these increased to large numbers during the succeeding week and then slowly increased throughout the experiment. In 2.53 the bacteria decreased over 99 per cent in 24 hours, and the numbers remained practically constant until the seventh day, when an increase started which lasted about two weeks, the numbers fluctuating and gradually falling off until the water became sterile on the 89th day. In 25.3 practically all of the bacteria were destroyed in 24 hours, small numbers being occasionally found until the 82d day, when the water became sterile. The bacterial results are shown in Table 5.

Experiment 176.—Water from a stagnant reservoir. Duration three days. Range 0.000253 to a.0253. The bacteria in the control and in 0.000253 decreased, while in 0.00253 and 0.0253 they increased, the greatest increase being noted in the bottle containing the most copper. The bacterial results are shown in Table 6.

TABLE 6.

Water from Stagnant Reservoir.

(Bacteria per c.c.)

Elapsed Time	Copper—Parts per 100,000			
	Control	0.000253	0.00253	0.0253
Start	120	...	...	...
1 day	110	210	400	700
2 days	34	55	1,900	19,000
3 "	13	31	1,800	12,400

Experiment 177.—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 0.0253. The bacteria in all of the bottles followed the same law, increasing rapidly during the first two or three days and then decreasing slowly. The bacterial results are shown in Table 7.

TABLE 7.

Water from Stagnant Reservoir.

(Bacteria per c.c.)

Elapsed Timf	Copper—Parts per 100,000			
	Control	0.00253	0.00506	0.0253
Start	3,900	......	......	......
1 day	29,400	22 000	24,500	24,500
2 days	16,900	22,900	90,200	144,000
3 "	10,100	16,400	40,000	165,600
5 "	6,800	4,100	21,000	10,500
7 "	5,000	4,700	4,000	6,900
9 "	4,200	10,500	32,500	90,000
12 "	270	420	3,600	2,500

Experiment 179.—Water from a stagnant reservoir. Duration eight days. Range 0.00253 to 2.53. The bacteria in the control fluctuated somewhat, but decreased throughout the experiment. In 0.00253 the numbers decreased during the first six

days and then showed an increase on the eighth day. In 0.0253 the numbers decreased during the first two days and then increased largely, the same being true with 0.253. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and continued to decrease to the sixth day, a large secondary increase being noted on the eighth day. The bacterial results are shown in Table 8.

TABLE 8.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start	300	....	.....	.....	.....
1 day	35	255	100	135	21
2 days	45	400	36	28	9
4 "	700	300	113,600	97,200	1
6 "	80	75	10,600	1,800	2
8 "	245	4,100	52,000	1,900	2,300

Experiment 187.—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 2.53. The bacteria in the control increased slowly throughout the experiment. In 0.00253 they increased during the first three days, and then fluctuated, but decreased throughout the remainder of the experiment. In 0.0253 over 90 per cent of the bacteria were killed in 24 hours, but the remainder increased rapidly throughout the experiment. In 0.253 the bacteria decreased during the first two days and then increased largely until the fifth day, a decrease following throughout the experiment. In 2.53 over 95 per cent of the bacteria were killed in 24 hours, the rest remaining practically constant, with the usual fluctuations. The bacterial results are shown in Table 9.

TABLE 9.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start	265	.....	....	....	..
1 day	230	210	16	95	9
2 days	1,500	2,500	1,300	65	0
3 "	425	11,000	10,400	23,200	2
4 "	1,260	720	38,800	442,800	0
5 "	1,118	7,200	48.400	630,000	..
7 "	4,500	700	1,900	241,900	3
9 "	8,900	865	51,000	75,600	5
12 "	15,500	3,200	24,000	240,000	8

Experiment 185.—Driven well water from Methuen town supply. Duration 133 days. Range 0.000253 to 0.253. There were only 31 bacteria in this water at the start. In all the samples a large increase was noted in 24 to 48 hours. The numbers in the control, 0.000253, and 0.00253 remained practically constant after 48 hours during 45 days, after which they began to decrease slowly. In 0.0253 a considerable decrease

occurred about the 84th day, the numbers remaining low after that time. In 0.253 the numbers were below 100 on the 31st day and continued low, with the exception of one count throughout the experiment. The bacterial results are shown in Table 10.

TABLE 10.

DRIVEN WELL WATER FROM METHUEN TOWN SUPPLY.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start	31	.....	.....	.....	.....
1 day	15,700	6,700	6,300	3,700	300
2 days	3,240	275	19,500	27,000	53,500
3 "	1,050	440	17,000	30,000	10,300
4 "	1,300	315	3,500	66,000	33,600
5 "	11,800	14,500	4,700	181,100	55,000
7 "	14,000	675	13,500	97,200	21,000
8 "	920	9,500	20,000	66,000	41,600
10 "	600	440	13,800	9,100	1,400
12 "	500	5,600	16,600	46,000	1,200
15 "	900	13,500	11,000	80,600	500
17 "	7,500	3,600	17,500	33,000	100
19 "	1,500	7,200	13,500	10,800	3,100
21 "	5,200	4,700	10,600	20,600	260
23 "	6,400	1,600	4,700	11,600	125
25 "	8,000	9.600	13,200	17,600	230
28 "	430	2,900	5,600	14,800	140
31 "	4,100	6,500	5,900	13,600	15
35 "	4,300	3,100	3,000	14,800	38
38 "	2,500	4,600	4,300	12,000	95
42 "	335	85	3,600	11,000	65
45 "	1,100	1,800	2,400	9,200	4
49 "	290	100	600	5,100	1
57 "	200	40	1,600	4,000	5
65 "	3,200	75	2,000	800	9
70 "	2,400	800	700	250	18
77 "	1,600	12	55	1,700	340
84 "	900	65	550	10	0
91 "	1,000	475	2,900	165	10
99 "	200	200	350	0	6
105 "	95	25	625	33	8
112 "	35	24	110	2	2
119 "	140	37	375	28	10
126 "	133	15	250	8	7
133 "	143	330	265	24	10

Experiment 164.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water. Duration six days. Range 0.000253 to 253. The B. coli in the control decreased during the first two days and then began to increase. In 0.000253 about

TABLE 11.

48 HOUR BROTH CULTURE B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	15,400	...	..	..	..	..	..	..
1 day	2,500	800	2	1	0	0	0	0
2 days	1,200	67	3	0	0	0	0	0
3 "	1,600	7	0	0	0	0	0	0
4 "	25,600	5	0	0	0	0	0	0
6 "	580,000	0	0	0	0	0	0	0

50 per cent were killed in 24 hours, the numbers decreasing rapidly until the water became sterile on the sixth day. In 0.00253 and 0.0253 all but a few of the germs were killed in 24 hours, and the water became sterile on the third and second days, respectively. In 0.253 to 253 inclusive all of the test organisms were killed in 24 hours. The detailed results are shown in Table 11.

Experiment 161.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water. Duration three days. Range 0.000253 to 253 inclusive. The test organisms disappeared from the control in 24 hours. In 0.000253 a large increase was noted in 24 hours. In 0.00253 about 90 per cent of the organisms were destroyed in 24 hours, and the water became sterile on the third day. In 0.0253 to 253 inclusive, like the control, the waters were sterile in 24 hours. The detailed results are shown in Table 12.

TABLE 12.

48 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. typhosus per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	3,100	.....	...	..	..	..	..	..
1 day	0	23,300	48	0	0	0	0	0
2 days	0	8,400	2	0	0	0	0	0
3 "	0	21,000	0	0	0	0	0	0

Experiment 191.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water. Duration 28 days. Range 0.000253 to 0.253. The test organisms in the control dropped off gradually from 115,000 at the start to only four organisms

TABLE 13.

24 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. typhosus per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start	115,500	......	......	.....	...
½ hour	71,000	234,000	14,500	6,500	520
1 "	100,800	90,000	195	1,000	24
2 hours	47,000	30,000	85	65	26
4 "	25,000	4,800	2	18	1
6 "	3,000	900	+*	2	+*
8 "	2,800	770	1	+*	+*
24 "	770	5	+*	1	+*
2 days	12	6,300	6,525	+*	+*
3 "	4	240	......	+*	+*
5 "	52,000	1,100	8	+*	+*
6 "	420,000	22,000	8	+†	+†
7 "	260,000	54,000	8	0‡	0‡
8 "	230,000	47,000	5	0‡	0‡
9 "	220,000	35,000	4	..	..
10 "	80,000	30,000	2	..	..
12 "	260,000	29,000	7	..	..
14 "	118,800	42,000	+*	..	..
16 "	86,400	36,000	+*	..	..
20 "	61,200	12,300	+*	..	..
22 "	72,100	24,200	+*	..	..
28 "	237,000	16,000	+*	..	..

*1 c.c.=0, 10 c.c.=+. †1–10 c.c.=0, 100 c.c.=+. ‡1, 10 and 100 c.c.=0.

on the third day, and then increased rapidly to over 400,000 on the sixth day, after which they decreased slowly throughout the experiment. The same phenomenon was noted in 0.000253, the bacteria dropping off to five individuals in 24 hours, after which they increased to over 54,000 on the seventh day, and then gradually decreased. In 0.00253 the organisms were destroyed rapidly, about 90 per cent being killed during the first hour, and in 24 hours it required 10 c.c. of the water to detect the test organism. On the second day, however, the organisms had increased, and small numbers were present until the 12th day. From the 14th to 28th days B. typhosus was not found in 1 c.c., but was present in 10 c.c. In 0.0253 over 90 per cent of the organisms were destroyed in 30 minutes, and nearly all had disappeared in six hours. From the second to fifth days the organism was found present in 10 c.c., and on the sixth day it was found present in 100 c.c., the water becoming sterile on the seventh day. In 0.253 over 95 per cent of the organisms disappeared in the first 30 minutes and the decrease was so rapid that after six hours it required 10 c.c. of the water to obtain a positive test. The organism was found continuously in 10 c.c. through the fifth day, and was found in 100 c.c. on the sixth day, the water becoming sterile on the seventh day. The detailed results are shown in Table 13.

Experiment 194.—24 hour broth culture of B. typhosus diluted 1:500 with sterile tap water. Duration 10 days. Range 0.0000253 to 2.53. In this experiment the typhoid bacilli were added to the water as a broth culture, and the water in the various bottles was allowed to stand four days before adding copper, the idea being that the organisms would have become in a measure inured to their new environment before they were subjected to the action of the copper sulphate. The numbers of B. typhosus

TABLE 14.

24 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:500 WITH STERILE TAP WATER AND ALLOWED TO STAND FOUR DAYS BEFORE ADDING COPPER SULPHATE.

(B. typhosus per c.c.)

ELAPSED TIME	BEFORE ADDING COPPER							
	A	B	C	D	E	F	G	H
Start...........	1,280,000	1,200,000	1,200,000	1,200,000	1,200,000	1,280,000	1,280,000	1,280,000
1 day...........	450,000	200,000	120,000	104,400	200,000	420,000	480,000	300,000
2 days..........	1,080,000	79,200	28,500	59,000	93,600	1,090,000	950,000	940,000
3 "	2,200,000	210,000	75,600	75,600	165,600	1,850,000	2,430,000	2,200,000
4 "	3,000,000	313,200	118,800	85,200	777,600	2,500,000	2,430,000	73,000

COPPER ADDED—PARTS PER 100,000.

Elapsed Time	Control	Duplicate Control	0.0000253	0.000253	0.00253	0.0253	0.253	2.53
30 minutes......	....	....	149,100	70,000	399,600	2,099,500	1,887,600	34,000
1 hour.........	....	....	183,600	239,000	529,100	1,120,000	1,358,500	27,500
2 hours........	....	....	159,800	72,000	626,400	1,657,500	640,000	2,225
4 "	....	....	175,000	149,000	748,400	4,406,400	323,800	142
6 "	....	....	205,200	109,400	1,101,600	2,814,000	48,800	15
24 "	3,400,000	902,900	604,800	376,000	3,700,000	4,200,000	4	1
2 days.........	2,750,000	2,000,000	1,040,000	1,980,000	2,520,000	2,750,000	0	0
3 "	....	3,000,000	1,120,000	1,200,000	2,200,000	2,450,000	0	0
4 "	265,000	4,400,000	330,000	14,400,000	4,500,000	3,000,000	0	0
8 "	2,200,000	4,600,000	10,300,000	9,360,000	3,100,000	2,060,000	....	.....
10 "	1,300,000	2,230,000	....	250,000	1,650,000	1,300,000	....	.....

during these four days increased in four of the bottles and decreased more or less in the other four. In the two bottles which were retained as controls the B. typhosus continued to increase during the 10 days they were under observation, the same being true of the waters containing copper 0.0000253, 0.000253, and 0.00253. In the water containing copper 0.0253 the numbers of B. typhosus remained practically constant during the 10 days. In 0.253 and 2.53 the numbers fell off rapidly, being practically all eliminated in 24 hours, and the waters becoming sterile in 48 hours. Tests were not made in volumes larger than 1 c.c. in this experiment. The detailed results are shown in Table 14.

EXPERIMENTS WITH METALLIC COPPER.

Experiment 175.—18.5 liters of Merrimac River water in copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 20 days. The bacteria in the control increased. The bacteria in the copper dish, with the exception of one slight increase, fell off gradually.

The B. coli in the control were found in 1 c.c. on the eighth day and in 100 c.c. on the 15th day. In the copper dish the B. coli disappeared from 100 c.c. on the third day. The bacterial results are shown in Table 15 and the B. coli results in Table 16.

Experiment 178.—18.5 liters of tap water containing 10 per cent of sewage in a copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 76 days. Copper absorbed 0.46 parts per 100,000 (87 days). The bacteria

TABLE 15.

(Bacteria per c.c.)

Elapsed Time	Experiment 175 Merrimac River Water		Experiment 178 Tap Water Containing 10 per Cent of Sewage	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start	1,400	1,400	320,000	390,000
1 day	9,300	1,500	1,950,000	1,440,000
2 days	11,000	17,300	2,980,000	3,430,000
3 “	488,500	337	470,000	2,690,000
4 “	1,114,000	1,600	350,000	250,000
6 “	704,900	3,100	12,000	420,000
8 “	16,200	95	12,000	69,000
10 “	107,000	180	1,230,000	210,000
13 “	125,000	85	110,000	30,000
15 “	307,000	85	73,400	26,500
17 “	414,000	30	77,800	39,000
20 “	1,490,000	37	300,000	39,000
22 ‘	....	....	73,000	20,000
24 “	....	....	240,000	4,000
28 “	....	....	180,000	12,300
30 “	....	....	230,000	5,000
32 “	....	....	180,000	4,500
34 “	....	....	118,800	2,800
36 “	....	....	29,000	3,000
38 “	....	....	168,500	2,700
41 “	....	....	55,000	2,500
44 “	....	....	86,400	2,400
48 “	....	....	101,500	3,000
55 “	....	....	174,200	1,100
62 “	....	....	93,600	200
70 “	....	....	172,800	100
76 “	....	....	350,000	3

TABLE 16.

(B. coli per c.c.)

Elapsed Time	Experiment 175 Merrimac River Water		Experiment 178 Tap Water Containing 10 per Cent of Sewage	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start	11	11	2,600	5,000
1 day	11	..	750	550
2 days	11	0†	700	100
3 "	+	0†	100	+
4 "	+	0†	+	+
6 "	+	0†	6	+
8 "	5	0†	+	3
10 "	+*	0†	1	6
13 "	+*	..	2	+
15 "	+*	..	0†	4
17 "	0†	..	0†	3
20 "	0†	..	0†	4
22 "	..	..	0†	0†
24 "	..	..	0†	0†

*1 c.c. = 0, 100 c.c. = +. †100 c.c. = 0.

in the control were practically constant throughout. The bacteria in the copper dish increased rapidly and then slowly decreased until the water was practically sterile on the 76th day.

The B. coli decreased rapidly in both cultures. In the control it was found in 1 c.c. on the 13th day, but disappeared from 100 c.c. on the 15th day. In the copper, B. coli were found in small numbers on the 20th day, five days after they had completely disappeared from the control. The bacterial results are shown in Table 15 and the B. coli results in Table 16.

Experiment 183.—18.5 liters of Merrimac River water in a copper dish, with control in an enameled dish. Exposed copper area 2,900 square c.c. Duration 71 days. Copper absorbed 0.30 parts per 100,000. Bacteria drop off gradually in both copper and control, both becoming practically sterile at end of experiment.

B. coli were found in 1 c.c. of the control on the 17th day and in 100 c.c. on the 28th day, disappearing on the 30th day. In the copper, B. coli were found in small numbers up to the sixth day, but disappeared from 100 c.c. on the eighth day. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

Experiment 200.—17.3 liters of Merrimac River water in a copper dish with control in an enameled dish. Exposed copper surface 2,900 square c.c. Duration 64 days. Copper absorbed 1.0 part per 100,000 (78 days). Bacteria in both control and copper dish decrease gradually, the decline in the copper dish being more rapid. The contents of the copper dish were sterile on the 55th day, and in the control only a few bacteria remained alive.

B. coli were found in the control in 1 c.c. as late as the 20th day, but disappeared from 100 c.c. on the 28th day. In the copper, B. coli disappeared from 1 c.c. on the fourth day, but were found in 100 c.c. as late as the 34th day, six days after they had disappeared from the control. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

TABLE 17.

(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 183 MERRIMAC RIVER WATER		EXPERIMENT 200 MERRIMAC RIVER WATER	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	600	1,200	11,000	10,500
½ hour	....	....	12,300	16,500
1 "	....	....	11,200	11,500
2 hours	800	500	10,800	7,300
4 "	700	490	11,000	7,200
6 "	400	135	12,000	6,000
8 "	800	200	20,000	3,800
24 "	1,300	65	16,000	104
2 days	1,044	120	6,500	400
3 "	7,700	....	12,000	200
4 "	103	763	1,200	42
6 "	164	133	5,500	133
8 "	245	156	5,400	40
10 "	250	110	12,800	15
13 "	275	25	300	7
15 "	180	12	....	....
17 "	792	13	....	....
20 "	20	10	1,200	15
23 "	2,590	9	....	....
28 "	100	13	46	9
30 "	260	13	....	....
34 "	82	10	28	2
42 "	90	12	11	0
48 "	10	6	65	3
55 "	3	1	6	0
64 "	3	4	2	0
71 "	5	6	....	....

TABLE 18.

(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 183 MERRIMAC RIVER WATER		EXPERIMENT 200 MERRIMAC RIVER WATER	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	32	44	...	...
½ hour	..	..	91	146
1 "	..	..	118	114
2 hours	48	10	118	97
4 "	23	12	120	96
6 "	48	+	74	63
8 "	44	12	105	15
24 "	30	+	52	+*
2 days	0	3	8	+*
3 "	25	4	8	11
4 "	7	+	+	+*
6 "	1	10	11	+*
8 "	11	0†	+*	+*
10 "	7	0†	+*	+*
13 "	1	0†	+*	+*
15 "	+	0†	..	..
17 "	+	..	..	..
20 "	+*	..	6	+*
23 "	+*	..	..	..
28 "	+*	..	0†	+*
30 "	0†	..	..	..
34 "	0†	..	0†	+*
42 "	..	..	0†	0†
48 "	..	..	0	0†

*1 c.c.=0, 100 c.c.=+.

†100 c.c.=0.

Experiment 182.—Three glass vessels containing respectively 18.9 liters of Merrimac River water, effluent from a sewage filter, and driven well water. 2,200 square c.c. of copper surface were inserted in each in the form of thin sheets. Duration seven days. Copper absorbed by river water 0.062, by sewage effluent 1.822, by driven well water 0.035. The bacteria in all samples increased. The bacterial results are shown in Table 19.

Experiment 186.—Duplicate of Experiment 182. Duration 14 days. Copper absorbed by river water 0.082, by sewage effluent 2.400, by well water 0.047. The numbers of bacteria in the river water showed only small fluctuations. In the sewage effluent the bacteria decreased about 85 per cent until the fifth day, and then increased until the 13th day. The bacteria in the well water increased to a maximum on the 10th day and then slowly decreased. The bacterial results are shown in Table 19.

TABLE 19.

WATER IN GLASS VESSELS CONTAINING SHEETS OF METALLIC COPPER.

(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 182			EXPERIMENT 186		
	Merrimac River Water	Effluent Sewage Filter	Driven Well Water	Merrimac River Water	Effluent Sewage Filter	Driven Well Water
Start	1,600	14,500	900	2,400	2,000	195
1 day	1,500	972	443	525	245	375
2 days	1,600	4,300	3,400	545	345	560
3 “	2,000	1,100	12.300	340	240	46,000
4 “	7,500	32.500	6,175	3,500	370	87,300
5 “	21,000	142,000	30,000	655	1,800	78,100
6 “	4,400	22,000	5,110	240	1,420	78,100
7 “	5,000	10,800	21,600	720	1,800	60.000
8 “	.....	.....	.....	900	11,200	43,000
9 “	.....	.....	.....	700	6,875	66,600
10 “	.....	.....	.....	6,000	12,000	100,000
11 “	.....	.....	.....	2,400	8,000	73,400
12 “	.....	.....	.....	585	63,000	70,000
13 “	.....	.....	.....	575	60,000	72,000
14 “	.....	.....	.....	3,200	4,750	69,000

Experiment 184.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration six days. Copper absorbed 0.50 parts per 100,000 (sterilized in dish and stood 12 days). B. coli in the control show a normal increase. In the copper over 99 per cent died out in 24 hours, but a few were alive on the sixth day. The detailed results are shown in Table 20.

Experiment 189.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with a control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration 12 days. Copper absorbed 0.68 parts per 100,000 (21 days). B. coli in control show a normal increase. In copper the numbers remained practically constant during five days, the test organisms being found in one c.c. on the eighth day, but having disappeared from that volume on the 10th day. The detailed results are shown in Table 20.

TABLE 20.

48 HOUR BROTH CULTURE OF B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 184		EXPERIMENT 189	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	370,000	350,000	430,000	360,000
2 hours	750,000	550,000	700,000	420,000
4 “	320,000	450,000	118.800	98,000
6 “	340,000	500,000	275,300	110,200
8 “	440,000	159,800	260,000	350,000
24 “	750,000	735	21,000	9,500
2 days	390,000	2	77,800	190,800
3 “	4,300,000	1	479,300	2,100,000
4 “	....	....	2,851,200	180,000
5 “	1,570,000	+	3,800,000	539,600
6 “	4,700,000	+	1,430,000	400
8 “	....	....	392,100	1
10 “	....	....	2,750,000	0
12 “	....	....	450,000	0

Experiment 199.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 19 liters of water. Copper surface 3,050 square c.c. Duration 62 days. Copper absorbed 0.54 parts per 100,000 (72 days). The numbers of B. coli in the control remained practically unchanged during 62 days. In the copper over 99 per cent of the organisms were killed in 24 hours, and it required 10 c.c. to give a positive for the organisms on the second and third days. On the fourth day a secondary increase commenced, and

TABLE 21.

48 HOUR BROTH CULTURE OF B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start	280,000	440,000
½ hour	280,000	380,000
1 “	380,000	290,000
2 hours	360,000	300,000
4 “	240,000	78,400
6 “	201,600	68,400
8 “	147,600	10,500
24 “	111,600	15
2 days	146,900	+*
3 “	1,026,000	+*
4 “	852,000	22
6 “	639,000	1
8 “	410,000	4,900
10 “	1,560,000	750
13 “	1,000,000	+*
20 “	170,000	+*
27 “	46,100	+*
33 “	229,000	+†
40 “	090,000	+†
47 “	400,000	0‡
54 “	520,000	0‡
62 “	100,000	0‡

*1 c.c.=0, 10 c.c.=+. †1 and 10 c.c.=0, 100 c.c.=+. ‡1, 10, and 100 c.c.=0.

on the eighth day the number of B. coli had increased to 4,900. On the 10th day a decline was noted. From the 13th to the 27th day the organism was found in 10 c.c., but disappeared from 100 c.c. on the 33d day. The detailed results are shown in Table 21.

Experiment 190.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface, 2,900 square c.c. Duration three days. The numbers of B. typhosus in the control decreased rapidly, over 99 per cent having disappeared in three days. In the copper the same rapid decrease was noted, over 99 per cent of the organisms having disappeared in six hours, and practically all in eight hours. After 24 hours the organism was not found in 1 c.c., but was detected in 100 c.c., and on the second day it had disappeared from 100 c.c. The detailed results are shown in Table 22.

TABLE 22.

48 Hour Broth Culture of B. typhosus Diluted 1:10,000 with Sterile Tap Water

(B. typhosus per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start	180,000	270,000
½ hour	200.000	93,600
1 "	47,000	39,000
2 hours	26,500	14,500
4 "	300	700
6 "	2,000	200
8 "	1,800	+
24 "	2,000	+*
2 days	330	0†
3 "	110	0†

*1–10–100 c.c.=0. †1–10 c.c.=0, 100 c.c.=+.

COMPARISON OF COPPER AND COPPER SULPHATE WITH OTHER METALS AND SALTS.

In the following digest of experiments, the various bottles of experiments with ferrous sulphate and aluminum sulphate, are designated by the amount of iron or aluminum present, in parts per 100,000.

Experiment 210.—Merrimac River water treated with ferrous sulphate. Range 0.002 to 2.01. Duration 131 days. The bacteria in the control increased during the first 24 hours and then gradually decreased until the 116th day, when a small secondary increase occurred. In .002 the increase in 24 hours was larger than in the control, after which the numbers decreased steadily throughout the period of observation. In 2.01 over 95 per cent of the bacteria were destroyed in 24 hours, but those remaining were able to increase steadily until the sixth day, after which a decline set in, lasting till the 109th day. A slight secondary increase began about the 116th day.

B. coli were found in gradually decreasing numbers in the control until the 95th day, and in .002 until the 88th day. In 2.01 the B. coli were nearly all destroyed in

TABLE 23.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start	17,000	......	......
1 day	72,500	123,500	500
2 days	64,800	86,400	1,300
4 “	35,000	13,300	8,500
5 “	10,700	10,500	7,300
6 “	17,500	36,000	83,000
7 “	12,000	12,500	6,550
8 “	2,200	61,400	......
11 “	11,000	10,800	26,500
13 “	8,900	1,200	9,500
15 “	8,000	3,700	9,500
18 “	6,200	5,000	7,400
21 “	3,300	900	2,200
25 “	600	1,600	1,440
32 “	390	110	350
39 “	190	205	410
46 “	110	245	305
53 “	95	90	260
60 “	65	90	95
67 “	75	110	46
74 “	170	75	250
81 “	21	57	28
88 “	140	110	85
95 “	120	12	67
103 “	38	8	18
109 “	55	100	80
116 “	1,100	180	700
123 “	1,100	375	275
131 “	500	170	1,200

TABBE 24.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(B. coli per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start	70	..	..
1 day	31	20	+
2 days	2	1	+
4 “	20	+	+
5 “	15	+	1
6 “	43	58	+*
7 “	12	+	+*
8 “	7	+	+*
11 “	12	3	+*
13 “	+	+*	+*
15 “	10	5	0
18 “	0	28	0
21 “	0	20	0
25 “	8	10	0
32 “	0	5	..
39 “	3	1	..
46 “	3	5	..
53 “	0	3	..
60 “	4	2	..
67 “	0	+*	..
74 “	2	1	..
81 “	0	+*	..
88 “	4	5	..
95 “	1	0	..
103 “	0	0	..

*1 c.c.=0, 10 c.c.=+ .

24 hours, the organism being detected in 1 c.c. up to the fifth day, and in 10 c.c. up to the 13th day. The bacterial results are shown in Table 23, and the B. coli results in Table 24.

Experiment 218.—Merrimac River water treated with ferrous sulphate. Range 0.020 to 20.1. The experiment was not complete when this was written.

TABLE 25.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start	600	.....	.....	.....	....
1 day	1,300	425	700	300	250
2 days	600	500	44	85	130
3 "	4,900	2,400	900	1,500	1,700
4 "	.....	.....	.....	.....	.....
5 "	20,000	12,900	8,800	2,200	9,100
7 "	7,700	2,000	9,700	1,400	400
9 "	7,500	2,000	1,600	2,000	50
11 "	2,100	300	1,400	15,400	0
15 "	3,800	375	275	2,000	18
18 "	2,200	1,400	800	2,800	600
21 "	300	230	70	1,100	14
28 "	140	250	120	600	5
35 "	230	75	47	325	23
42 "	16	50	130	190	11
49 "	38	70	35	65	29
56 "	21	51	47	31	16
63 "	11	10	50	22	37
70 "	35	12	36	17	52
78 "	46	12	32	11	30
84 "	108	65	37	72	30
91 "	15	26	19	49	28

TABLE 26.

MERRIMAC RIVER WATER.

(B. coli per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start	20	..	..	..	..
1 day	26	15	6	2	3
2 days	8	23	9	6	3
3 "	19	9	15	20	4
4 "	7	4	6	11	4
5 "	3	4	0	2	3
7 "	0	7	0	0	8
9 "	3	0	2	14	0
11 "	4	0	5	..	0
15 "	11	16	6	6	7
18 "	0	1	2	10	0
21 "	0	2	0	0	0
28 "	0	0	0	0	0
35 "	15	6	3	0	0
42 "	12	0	4	11	3
49 "	3	3	1	2	5
56 "	7	9	0	1	1
63 "	1	4	0	0	0
70 "	10	5	16	16	10
78 "	8	11	4	6	1
84 "	0	0	0	1	0
91 "	0	15	14	17	3

The bacteria in all the bottles increased steadily for a few days, then decreased slowly. The maximum was reached in the control, 0.020, and 20.1 on the fifth day, in 0.201 on the ninth day and in 2.01 on the 11th day. B. coli were found in small numbers as late as the 78th day in the control, on the 91st day in the waters which had been treated. The bacterial results are shown in Table 25 and the B. coli results in Table 26.

Experiment 158.—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration four days. The bacteria in all samples were reduced during the first 24 hours, and then began to increase about the third or fourth day. The results are shown in Table 27.

TABLE 27.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start	950	.....	.....	.....	.....
6 hours	800	20	255	110	145
1 day	205	143	120	55	95
2 days	.....	248	176	155	70
3 "	1,200	400	790	10	2,800
4 "	.....	2,800	3,800	20,000	4,700

Experiment 165.—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration 11 days. The bacteria in the control increased to a maximum on the third day and then declined. In all of the treated samples the bacteria were much reduced on the second day, but all showed a material increase from the third to the seventh days.

The B. coli results in this experiment are interesting, showing the occasional appearance of considerable numbers in all treated samples at intervals, with intermediate periods when the test organism was not detected. This phenomenon was probably due to errors in sampling caused by the precipitated aluminum hydrate, and would indicate that the B. coli were able to live in the precipitate and perhaps to increase. The bacterial results are shown in Table 28 and the B. coli results in Table 29.

TABLE 28.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start	1,450	.....	.....	.....	.....
1 day	176,400	4,900	3,200	800	1,900
2 days	321.900	550	290	110	125
3 "	430,000	11,200	11,400	1,050	155
5 "	129,000	25,800	4,600	1,700	260
7 "	7,700	3,200	40,000	5,900	75,600
9 "	15,500	1,600	26,300	3,000	25,500
11 "	16,500	18,500	30,500	41,400	28,200

TABLE 29.

MERRIMAC RIVER WATER.

(B. coli per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start................	36	..	..	..	..
1 day..............	6	0	0	0	0
2 days.............	95	11	20	0	25
3 "	65	1	16	0	0
5 "	20	0	0	22	0
7 "	25	0	0	0	0
9 "	11	35	63	40	114
11 "	0	0	0	21	0

NOTE.—The occasional appearance of B. coli was probably caused by imperfect sampling due to the precipitated hydrate of alumina.

Experiment 209.—1,000 c.c. of Merrimac River water were placed in each of six jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six jars respectively, the seventh being retained as a control. Exposed metal surface 625 square c.c. Duration 132 days. The bacteria in the control showed a steady decrease after the first 24 hours. With the copper, the bacteria decreased during the first 24 hours, and then increased to a maximum on the eighth day, dropping

TABLE 30.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start..............	10,000	75,600	14,600	18,000	9,000	75,600	7,700
4 hours..........	11,000	700	11,000	12,700	4,100	15,200	18,000
8 "	19,200	180	15,500	9,300	6,200	24,000	14,700
24 "	9,700	430	8,500	2,900	5,200	14,000	5,500
2 days..........	2,500	75,600	7,000	4,500	11,000	6,700	7,300
3 "	800	850	5,000	24,000	14,500	4,300	1,400
4 "	800	......	1,200	33,000	5,500	1,750	600
6 "	1,000	360,000	600	30,800	28,500	1,700	65
8 "	1,450	673,000	900	38,300	4,050	900	62
10 "	250	97,200	150	2,000	650	700	29
13 "	110	181,500	120	2,000	950	50	20
15 "	125	108,000	65	21,400	450	55	80
17 "	23	51,000	100	21,250	65	105	300
20 "	250	59,000	85	22,500	7	45	110
23 "	180	31,500	9	22,100	10	90	230
27 "	43	0	8	800	0	43	10
34 "	....	310	55	1,100	0	12	360
41 "	180	10	10	1,200	....	43	175
48 "	290	4	100	490	....	720	430
55 "	225	2	35	400	....	770	60
62 "	260	4	5	130	....	60	60
69 "	200	0	0	55	....	70	80
76 "	160	2	3	20	....	550	42
83 "	350	10	0	10	....	375	47
90 "	150	9	26	6	....	120	110
97 "	125	4	12	14	....	210	220
105 "	35	4	55	180	....	350	130
111 "	75	30	65	500	....	350	75
118 "	23	0	40	7	....	75	36
125 "	38	13	60	19	....	260	100
132 "	5	7	3	22	....	52	85

TABLE 31.

MERRIMAC RIVER WATER.

(B. coli c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start	32	52	18	19	5	4	25
4 hours	18	47	38	14	35	32	15
8 “	1	2	+	1	7	6	+
24 “	12	3	+	+	+	10	6
2 days	10	2	10	3	3	3	+
3 “	+	+	10	+	5	+	+
4 “	1	+	+	1	+	+	1
5 “	5	4	6	12	+	4	+
8 “	26	10	10	19	6	25	0
10 “	9	3	+	+	0	9	0
13 “	6	+	+	2	0	3	6
15 “	1	+	2	3	0	3	0
17 “	7	+	2	+	0	6	0
20 “	8	+	3	10	0	16	0
23 “	+	+	21	4	0	10	0
27 “	3	4	9	2	0	13	0
34 “	2	9	6	5	..	2	0
41 “	5	3	0	3	..	0	0
48 “	+	0	0	+	..	0	0
55 “	2	0	0	12	..	0	0
62 “	4	0	0	7	..	0	0
69 “	+	0	0	+	..	0	0
76 “	1	..	..	1	..	..	..
83 “	0	..	..	+	..	..	..
90 “	0	..	..	3	..	..	..
07 “	..	..	..	0	..	..	..
105 “	..	..	..	0	..	..	..

off slowly till the 23d day. With the aluminum there was a steady decrease in bacteria throughout the experiment. With the lead, the bacteria increased slightly to a maximum on the eighth day, remaining practically constant till the 23d day, after which they decreased steadily, with a slight secondary increase on the 105th day. With the zinc the bacteria increased slightly to a maximum on the sixth day and then declined rapidly, the water becoming sterile on the 27th day. The bacteria decreased steadily in the tin until the 41st day, when a small secondary increase began, lasting until the 125th day. With the iron, the bacteria decreased steadily, with small secondary increases from the 17th to the 48th days, and from the 90th to the 105th days.

The B. coli in all the samples decreased steadily, with the usual fluctuations, and disappeared from the zinc on the 10th day, from the iron on the 15th day, from the tin and aluminum on the 41st day, and from the copper on the 48th day, from the control on the 83d day, and from the lead on the 97th day. The bacterial analyses are given in Table 30 and the B. coli determinations in Table 31.

Experiment 237.—1,100 c.c. of Merrimac River water containing 1 per cent of sewage was placed in each of seven jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six of the jars respectively, the seventh being retained as a control. The exposed metal surface in each case was 625 square c.c. The experiment had only run 31 days at the time this was written. In the control, lead, and tin, the bacteria decreased steadily throughout the period of observation. In the copper a large increase occurred during the first four days, after which the bacteria declined steadily. In the aluminum the bacteria decreased steadily until the 15th day, when a secondary increase started lasting till the 23d day. With the zinc a considerable reduction occurred during the first 24 hours, after which the numbers increased till the third day, when a decline set in lasting throughout the period of observation. In the iron an increase occurred during the first 24 hours, after which a steady decrease was noted until the 15th day, when a secondary increase commenced which lasted until the 20th day.

The numbers of B. coli in the control increased largely during the first 24 hours, after which they decreased steadily until the water was practically sterile after 31 days. In all of the jars containing metals the B. coli decreased steadily, a slight secondary increase being noted on the 17th day in the aluminum. The fluctuations in the numbers of B. coli were quite noticeable, in a number of instances no organisms being found in one c.c. for a number of days, and then appearing in small numbers. The B. coli disappeared from the copper on the sixth day, from the zinc on the 10th day, from the tin on the 15th day, from the iron and lead on the 23d day, and probably from the aluminum on the 31st day. The bacterial analyses are given in Table 32 and the B. coli determinations in Table 33.

TABLE 32.

Merrimac River Water Containing 1 per Cent of Sewage.

(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start	44,500	.....	.....	.....	.....	.....	.....
1 day	21,000	127,800	44,000	56,000	7,000	35,000	86,600
2 days	150,000	362,100	16,500	26,000	13,400	12,000	16,500
3 "	11,000	180,000	2,800	8,500	16,500	1,400	35,000
4 "	4,600	305,000	11,500	8,500	10,500	4,800	18,000
6 "	415	35,000	310	5,500	11,200	200	205
8 "	2,800	96,300	220	3,500	3,700	138	93
10 "	52	3,600	95	3,000	19	94	63
13 "	69	6,700	55	155	115	18	8
15 "	17	245	1,000	80	21	27	105
17 "	23	790	7,600	3	100	105	760
20 "	31	115	3,000	9	9	235	1,500
23 "	5	100	3,850	18	40	275	525
28 "	10	65	480	20	5	210	30
31 "	25	275	325	135	116	160	125

TABLE 33.

Merrimac River Water Containing 1 per Cent of Sewage.

(B. coli per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start	3,500	3,500	3,500	3,500	3,500	3,500	3,500
1 day	38,900	2	1,370	149	22	3,100	650
2 days	250	....	200	0	0	100	50
3 "	108	12	75	3	3	45	230
4 "	25	25	21	12	2	20	125
6 "	20	0	9	1	0	8	20
8 "	13	2	8	0	4	7	5
10 "	15	0	0	0	0	0	0
13 "	3	0	5	5	0	6	1
15 "	1	0	0	2	0	0	1
17 "	1	0	125	7	0	1	25
20 "	0	0	45	1	0	1	6
23 "	4	0	7	0	0	0	0
28 "	1	0	3	0	0	0	0
31 "	0	0	0	0	0	0	0

RÉSUMÉ.

In the following résumé, the results of the copper sulphate experiments are brought together according to the amount of copper added to the water. This copper is expressed as the ratio of copper sulphate by weight to the weight of water, with the equivalent of metallic copper in parts per 100,000 in parentheses.

Controls.—In three experiments with polluted water, one showed a gradual decrease in bacterial contents, and the other two showed an increase, then a decrease which was followed by a secondary increase after some time. In four experiments with stagnant water, run for a short time only, one showed a decrease, one increased, one remained practically constant, and one increased and then decreased. In one experiment with driven well water, the bacteria increased, then decreased, and continued to fluctuate up and down during the 133 days the experiment continued.

In the one experiment made, B. coli under natural conditions showed a gradual decrease, but continued alive in 1 c.c. during 95 days. B. coli added to water as broth culture decreased, then increased. In three experiments using laboratory cultures of B. typhosus, the control was sterile after 24 hours in one case; in another a sharp decrease was noted till the third day, when an enormous increase commenced which lasted through 28 days; and in a third case the typhoid bacilli, which were present in large numbers, remained practically unchanged during 14 days.

1: billion (0.0000253).—In the one experiment made the bacteria followed the same laws as did the control.

1:100 million (0.000253).—The behavior of the normal water bacteria was observed in five experiments, four with polluted water and one with stagnant water. In four of the experiments the bacteria followed the same curve as in the control, and in the fifth they were nearly all destroyed at first, but the few remaining were able to multiply to large numbers.

In one experiment, B. coli, naturally present, acted much like the control, decreasing slowly but remaining alive some 88 days in 1 c.c. In one experiment with a laboratory culture a rapid decrease occurred, and the B. coli had disappeared at the end of six days.

Three experiments were made with laboratory cultures of B. typhosus. In one experiment large numbers survived three days, although the control died out in 24 hours; in another nearly all the B. typhosus were killed in 24 hours, but the very few remaining were able to increase to large numbers. In the third experiment the B. typhosus had become somewhat accustomed to life in water before the copper was added, and increased steadily during the 10 days they were under observation.

1:10 million (*0.00253*).—The normal bacteria were observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. Three of the polluted waters acted like the control, but in the other nearly all the bacteria were destroyed at first, the few remaining, however, being able to increase rapidly. In the three stagnant waters the bacteria increased, although they failed to do so in one of the stagnant water controls.

Natural B. coli in one experiment decreased slowly, as they did in the control, but the numbers were usually larger than in the control. In one experiment with a laboratory culture the B. coli were killed in three days.

Two experiments were made with laboratory cultures of B. typhosus; in one the test organisms disappeared from 1 c.c. in three days, and in another nearly all were killed in four hours, but some remained alive in 1 c.c. till the 12th day, and tests of 10 c.c. showed them to be alive up to the 28th day. In one experiment with a culture of B. typhosus which had been grown four days in water, a steady increase occurred during the 10 days it was under observation.

1: million (*0.0253*).—The behavior of the water bacteria was observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. In two of the polluted waters the bacteria followed the control, while in the other two they increased. In the three stagnant waters the bacteria increased, although they decreased in one of the controls. The bacteria in the well water followed the control.

Natural B. coli in one experiment decreased slowly, as did the control, but showed higher numbers than in the control. In one experiment with a laboratory culture, the B. coli were killed in two days, testing 1 c.c.

In one experiment with a laboratory culture of B. typhosus the organism disappeared from 1 c.c. in 24 hours. In another experiment the B. typhosus were nearly all destroyed in six hours, a few were alive on the fifth day, but none were found in 100 c.c. on the seventh day. In the experiment where the typhoid bacilli were inured to the water, the numbers increased steadily during the 10 days they were under observation.

1:100,000 (0.253).—The bacteria were observed in seven experiments, four with polluted water, two with stagnant water, and one with deep well water. In three of the polluted waters nearly all of the bacteria were killed out at first, but the few remaining were able to multiply largely. In the other polluted water a large increase occurred at once. The numbers of bacteria in the two stagnant waters remained low for two days and then increased rapidly. In the well water the bacteria followed the same curve as in the control.

Natural B. coli followed the same curve as the control. With a laboratory culture of B. coli the organism disappeared from 1 c.c. in 24 hours. In one experiment with B. typhosus the organism disappeared from 1 c.c. in 24 hours. In another experiment all but a few were killed in six hours, but 10 c.c. tests showed some to be alive on the fifth day, and 100 c.c. tests showed some alive on the sixth day. In the experiment with water-grown typhoid, the organisms disappeared from 1 c.c. in 24 hours.

1:10,000 (2.53).—The total bacteria were observed in six experiments, four with polluted water and two with stagnant water. In two of the polluted waters the bacteria decreased gradually, and the waters became practically sterile after 89 and 131 days respectively. In one experiment nearly all of the bacteria were killed immediately, and in another the bacteria were practically all destroyed at first, but a few remained during 20 days. The bacteria in the two stagnant water experiments were practically all destroyed in 24 hours.

Natural B. coli acted like the control and remained alive in 1 c.c. after 103 days. With a laboratory culture of B. coli the organism disappeared from 1 c.c. in 24 hours. A laboratory culture of B.

typhosus and the special water culture of the same organism both disappeared from 1 c.c. in 24 hours.

1:1,000 (25.3).—The behavior of the normal bacteria was observed in three experiments with polluted water. Nearly all of the bacteria were destroyed in a short time in all of these experiments, but a few remained alive for 10 days, 20 days, and 68 days, respectively.

With a laboratory culture of B. coli and one of B. typhosus the organisms disappeared from 1 c.c. in 24 hours.

1:100 (253).—Two experiments were made with polluted water, in which all of the bacteria were killed in 24 hours.

The complete sterilization of water by allowing it to stand in a clean copper dish does not seem to be an accomplished fact; in only one of six experiments was the water completely sterilized, and that only after standing 55 days. The bacterial curves, taking both controls and copper dish cultures, seem to follow the same laws hitherto noted for standing water experiments. In two experiments the copper dish cultures consistently decreased in bacterial contents throughout, while in another experiment the numbers increased sharply and then declined slowly, both of which phenomena have been frequently observed in the various control cultures. In two experiments three entirely different waters were under comparative observation. The bacterial contents of each of these waters increased constantly, although one of them absorbed relatively large amounts of copper.

In four of the copper dish experiments, determinations of B. coli and of bacteria were made simultaneously. In these experiments the B. coli were present naturally, i. e., they came from fecal matter directly polluting the water, as opposed to experiments in which B. coli were added to the water in the form of a laboratory culture. Naturally we should expect to find the organisms under such conditions more resistant than in the case where we are dealing with cultures, and this proves to be the case. In one experiment the test organism disappeared from the water, testing 100 c.c. on the third day. In two other experiments the B. coli died out in the control dishes before they disappeared from the waters contained in copper, being found in 1 c.c. in one experiment on the 20th day, or five days after they had disappeared from 100 c.c. of the control; and in the other experiment they were found in 100 c.c. on the 34th day, or six days after they had disappeared from a like volume of the control.

Three experiments were made with laboratory cultures of B. coli in sterile water standing in copper, and one experiment was made with B. typhosus under the same conditions. In two experiments B. coli were found in 1 c.c. on the sixth and eighth days, respectively, tests not being made in larger volumes in these experiments. In another experiment B. coli appeared in some numbers as late as the 10th day, and were found in 100 c.c. as late as the 40th day. In the experiment with a typhoid the bacilli were practically all destroyed after eight hours, and were not found in 100 c.c. after 24 hours.

From the two experiments made with sulphate of alumina and ferrous sulphate, these two salts appear to have about the same action on the bacteria and B. coli as have equal strengths of copper sulphate.

Judging from the results of experiments comparing metallic copper with other metals, all of the metals tested seem to be about equal in their effect on the numbers of bacteria in waters with which they are in contact. In one experiment the water in contact with zinc became sterile after about three weeks, while the numbers of bacteria increased in the water which was in contact with copper, and with all the metals, excepting zinc, a few bacteria were alive after 132 days.

Based on the disappearance of B. coli in 1 c.c. the metals in one experiment ranked:—zinc 10 days, iron 15 days, tin 41 days, aluminum 41 days, copper 43 days, lead 97 days; and in the other experiment:—zinc 10 days, copper 10 days, tin 23 days, iron 23 days, lead 23 days, aluminum 31 days.

CONCLUSIONS.

In conclusion, the writers believe that the treatment of water with copper sulphate or by storing it in copper vessels has little practical value, for the following reasons:

I. The use of any method of sterilization which is not absolutely effective is dangerous in the hands of the general user, tending to induce a feeling of false security, and leading to the neglect of ordinary precautions which would otherwise be employed.

II. The removal of bacteria, B. coli and B. typhosus, by allowing a water to stand in copper vessels for short periods, while occasionally effective, is not sure, and the time necessary to accomplish complete sterilization is so long that the method would be of no practical value to the ordinary user. Furthermore, metallic copper seems to have little more germicidal power than iron, tin, zinc, or aluminum.

III. Although the removal of B. coli and B. typhosus is occasionally accomplished by dilute solutions of copper sulphate, these organisms may both live for many weeks in water containing copper sulphate in greater dilutions than 1:100,000; and in order to be safe dilutions of 1:1,000 must be used, in which case the water becomes repugnant to the user because of its strongly astringent taste.

IV. In some instances very dilute solutions of copper sulphate or colloidal copper absorbed from contact with clean metallic copper, appear to have a decidedly invigorating effect on bacterial activity, causing rapid multiplication, when the reverse would have been true had the water been allowed to stand the same length of time without any treatment.

NOTES IN REGARD TO THE DETERMINATION OF COPPER IN WATER.

FRED B. FORBES AND GILBERT H. PRATT.

IN the course of a series of experiments carried on by the Massachusetts State Board of Health it became necessary to devise a method for separating and determining quantitatively small amounts of copper in water; such a method has been worked out and is published in detail in the "Standard Methods of Water Analysis" of the Laboratory Section of this Association.[1] It is the purpose of this short paper to give a brief outline of the method, together with some experimental results, and to call attention to certain statements that have appeared in print regarding the total disappearance of the copper in a few hours when applied to a water supply, and the impossibility of detecting it in the water by chemical tests.

The chemical test in general use for detecting copper in solution in small quantities is that mentioned by Moore and Kellerman in *Bulletin 64* of the Bureau of Plant Industry, and consists of adding potassium ferrocyanide to the solution to be tested, acidified by acetic acid. The sensitiveness of this test is shown by the following figures.

.00001 gm. copper, as copper sulphate, in 20 c.c. distilled water, equivalent to 1 part copper in 2,000,000, gave a very faint color, which it would be impossible to identify as the characteristic red of the reaction. 0.00002 gm. copper in 20 c.c. water, equivalent to 1 part copper in 1,000,000, gave distinctly the characteristic color. Both these strengths of copper solution were tested also in volumes of 100 c.c. in tubes, in order to observe the colors through a longer column than in the previous experiments, but with the same results.

The above tests were made with a solution of copper sulphate in distilled water, no other substances being present. It is well known that the presence of iron seriously interferes with the test, and it has been found that the coloring matter of natural waters is also a troublesome factor, so that in practice the sensitiveness of the test is much less than is indicated by the figures obtained with solutions in distilled water. A considerable quantity of water can be concentrated to small volume in order to increase the value of this test, but it then becomes necessary to provide for the removal of iron and organic coloring matter, or the results obtained are of doubtful significance.

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[1] *Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 1.

Among various published accounts of the treatment of water supplies with copper sulphate, the writers have noticed in several cases the statement that after a few hours no copper could be detected in the water by the most delicate chemical tests, but have failed to see in any instance an account of the manner of making these tests, or any figures to prove their accuracy.

Owing to the unreliability of these qualitative tests for very small amounts of copper in natural waters, and to the necessity of obtaining quantitative results for purposes of comparison, it has been found that the only satisfactory way is to concentrate a large quantity of the water under examination, separate out the copper in proper condition for electrolysis, and deposit it as metal on a platinum electrode and weigh it.

The amount of water which is concentrated depends upon the amount of copper suspected to be present. Such an amount as will yield a weight of from 5 to 10 milligrams of metallic copper is preferable, as this amount of copper adheres well to the inside of the platinum dish which serves as an electrode, and can be weighed with accuracy on an analytical balance.

If the amount of copper in the water is very small, however, it is necessary to be content with a less weight, in order to avoid concentrating a very large volume of water.

It is the custom in the work of our board to collect three five-gallon carboys of water for each sample. Of this, 50 liters are concentrated for analysis, an equal amount being taken from each carboy and boiled down in three 10- or 14-inch porcelain dishes, with the addition to each dish of a little hydrochloric acid and 5 or 10 c.c. nitric acid. When concentrated as far as possible, the residues are united in one small dish, nitric acid being used to rub off the scum of organic matter which adheres to the sides of the dishes, and after further concentration the whole is treated with strong sulphuric acid and fumed over a lamp for some minutes. By this process, all silica is rendered insoluble, much of the organic coloring matter is destroyed, and the metals present are converted to sulphates. After dilution and filtering, the excess of sulphuric acid is neutralized, and hydrogen sulphide added to the sulphuric acid solution. By this means the rest of the coloring matter is left behind, together with zinc, if present, and practically all of the iron. Lead, if present, will have been previously removed as sulphate. The sulphides are dissolved in nitric acid, converted to sulphates by strong sulphuric acid, and any iron remaining is removed by ammonia. The filtrate from the iron is acidified with sulphuric acid and, as a precaution, is allowed to stand for some time, in which case any lead which remained after the two previous filtrations will deposit as sulphate. The solution is then treated with 10 c.c. of concentrated sulphuric acid and 1 gram of urea and placed in a platinum dish and electrolyzed, the inside of the dish becoming the cathode, while a coil of platinum wire is placed in the solution for the anode. Any zinc which might be present remains in solution; in fact, we have made use of this method for separating quantitatively considerable amounts of zinc from small amounts of copper, or *vice versa*.

When the copper is all deposited, the dish is washed, dried and weighed; then the copper is dissolved off the dish in dilute nitric acid, and the dish dried and weighed again. The difference in weight gives the weight of copper. There is occasionally a very small amount of organic matter, due to the decomposition of the urea or organic matter in the solution adhering to the dish, and insoluble in nitric acid; hence the manner of weighing, instead of taking the weight of the empty dish before the electrolysis.

The solution of the copper in nitric acid may be made ammoniacal, when the characteristic blue color due to copper will appear. It has been found that 0.0001 gm. of copper in a volume of 20 c.c. gives a faint blue color by this test, while 0.0002 gm. copper gives a very distinct color. This represents a sensitiveness of 1 part in 200,000 and 1 part in 100,000, respectively. A set of standards may be made up with known amounts of copper in this condition and the copper obtained by electrolysis can be read on such standards, though 0.0001 gm. copper is the smallest amount that can be detected; and it is impossible to interpolate between successive 10ths of a milligram. The test is of service, however, in verifying the result when the weight of copper obtained is only a few 10ths of a milligram.

Following are a few results obtained by the method with standard solutions, and also with natural waters to which known amounts of copper were added. In every case a considerable quantity of water was treated with the copper solution and then concentrated and carried through in the same manner as an unknown.

TABLE 1.

Experiments with Solution of Copper Sulphate in Distilled Water.

Weight of Copper Taken (Gram)	Weight of Copper Found (Gram)
0.0006	0.0005
0.0009	0.0007
0.0021	0.0019
0.0047	0.0050

TABLE 2.

Experiments with a Natural Surface Water to Which Known Amounts of Copper Sulphate Were Added.

Weight of Copper Taken (Gram)	Weight of Copper Found (Gram)	Quantity of Water Concentrated (Liters)
0.0006	0.0008	50
0.0014	0.0017	50
0.0047	0.0048	34
0.0091	0.0085	34
0.0222	0.0202	16
0.0404	0.0360	16

These results show the method to be capable of separating and determining small amounts of copper with considerable accuracy. In studying the figures it appears that the percentage error is quite large, especially in the case of the smaller quantities. It must be borne in mind, however, that the absolute error is very small; in fact, in some instances as small as the analytical balance is capable of detecting. In the case of the larger quantities, an additional error is introduced by dividing the solution before electrolysis, in order to avoid having too heavy a deposit on the dish. All of the results were obtained without previous knowledge by the operator of the amounts of copper present, and the determinations were carried out in all respects as with unknowns.

TABLE 3.

This table shows a series of results obtained from samples of water from a large natural reservoir infested with algæ, to which copper sulphate had been applied. In every case 50 liters of water were taken for analysis; the figures show the weight of copper found in each sample and its approximate dilution.

	Gram		
Before dosing	0.0001	Equivalent to 1 part Cu in	500,000,000
1 day after dosing	0.0025	“ “ 1 “ “ “	20,000,000
2 days “ “	0.0016	“ “ 1 “ “ “	31,250,000
3 “ “ “	0.0012	“ “ 1 “ “ “	41,666,000
7 “ “ “	0.0006	“ “ 1 “ “ “	83,333,000
13 “ “ “	0.0004	“ “ 1 “ “ “	125,000,000
27 “ “ “	0.0002	“ “ 1 “ “ “	250,000,000

These figures show clearly the necessity of operating on considerable quantities of the sample if reliable quantitative results are to be obtained. They show also the fallacy of depending on the ordinary qualitative tests to prove a water free from copper. The most delicate of the various tests would fail to reveal the presence of the metal in the original concentration in any of the foregoing samples, while if the water is subjected to concentration, some provision must be made for the removal of iron and organic coloring matter, or the accuracy of the test is vitiated.

It is believed that the method as devised is the most accurate and satisfactory one at the present time, and, though somewhat tedious, justified by the importance of thoroughly testing this comparatively new process of water purification before passing judgment on its value.

The method may also be used upon sand, the copper being extracted therefrom by means of nitric acid and the subsequent procedure being the same as with a water residue.

A NOTABLE SOURCE OF ERROR IN TESTING GASEOUS DISINFECTANTS.

Hibbert Winslow Hill.
Minnesota State Board of Health Laboratories, Minneapolis, Minn.

The testing of gaseous disinfectants for public health purposes is done for two general objects, in two different ways. The first object is that sought when the investigator determines from a large number of experimental tests the required amounts of gas and best methods of work, and then prescribes these for use in practice. The second object is to secure a check system by which each particular disinfection performed in practice is tested as a routine procedure by the exposure in the room of one or more test organisms, the death of which is required before the disinfection is officially approved. The two systems supplement each other, but the second is scarcely required if the disinfection is performed strictly according to a proper prescription, and is intended primarily as a test of the disinfector rather than of the disinfectant. For whatever purpose the tests are made, it is rather obvious that they should be so designed that the survival of the test organism should be good evidence that the room treated would have remained infected, had it been originally infected naturally by a patient suffering from an infectious disease, and also—*vice versa*—that the death of the test organism should prove that the room treated would no longer be infective, if it were infective before disinfection was done. It is of the greatest moment, therefore, that the condition of the test organisms, as well as their character, should parallel closely, or, if possible, be identical with, the condition of the infective agents which are, or are supposed to be, present in rooms occupied by infected patients.

The writer has had occasion to point out in previous articles[1] the probable position and condition of infective agents in naturally infected rooms. It is now his object to point out, from accumulated evidence, the requisites which should be demanded of test organisms used in testing disinfectants, these requisites being based on the premise above given, i.e., that the test organism should parallel in condition, as well as character, the infective agents which it is desired to destroy.

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[1] *Am. Pub. Health Assoc. Rep.*, 1902, 28, pp. 209, 509; *Bulletin*, Vermont State Board of Health; presented at the Vermont summer school for health officers, 1903.

It has been pointed out by a number of investigators that the efficiency of gaseous disinfectants, chlorine, sulphur, formaldehyde, carbolic acid vapor, etc., is dependent very largely on the humidity of the atmosphere in which they act. In saturated atmospheres remarkably small amounts of these gases are efficient. In dry atmospheres, they are practically inert, even if present in relatively large quantities. But a most important point is this, that a dry gas, acting upon a moist organism, kills it just as surely as does a moist gas acting upon a dry organism. This very simple fact has led to much of the confusion of results in work carried on by different observers, who, attempting to obtain similar results by similar methods, obtained contradictory results because of the differences in the degree of moistness of the test organisms respectively used; while another set of contradictions has resulted from differences in the humidities of the atmosphere in different tests. With two such important variables almost entirely overlooked in most "practical" disinfection tests of gaseous disinfectants, it is not astonishing that one observer records excellent results from the use of a method which, in the hands of another, utterly fails.

The work upon which this paper is based was done in the attempt to reconcile two sets of absolutely contradictory results obtained, one set in Boston, the other in the hands of a high authority not far away from Boston. A third, and more unusual, source of error was incidentally discovered—the drying of test organisms to an unusual extent; the discovery resulting finally in a statement from one observer that the sum total of favorable results he had so far obtained was absolutely worthless, since his controls themselves did not survive the period of drying employed, without the use of any disinfectant at all.

Briefly summed up, the facts are that test organisms, fresh and moist, are very susceptible to small quantities of disinfectant gas, dry or moist; the same organisms, fresh but dried, are extremely resistant to the same amounts of dry gases; while, finally, the same organisms, dried for a week or more, lose vitality to such an extent that even small amounts of gas, not necessarily at high humidities, will kill them. The writer has exposed in the same room, under identical conditions and at the same time, the same organisms on filter paper, and kept all night, one-half in such a manner as to dry thoroughly, the other half in such a manner as to remain moist. The moist organisms were all killed, the dry organisms all survived. Test objects prepared so that an intermediate stage of dryness was reached, behaved irregularly, some surviving, some perishing. Moreover, organisms (B. pyocyaneus) prepared on successive days, in such a manner that at the end of a week, all could be exposed under identical conditions to the same gas, gave results which showed that those prepared immediately before the exposure (i. e., still moist) were killed, those prepared two, three, and four days before (i. e., well dried, but still fresh) survived; while those prepared five, six, and seven days before were killed in numbers proportionate roughly to the age of the specimen, those longest dried showing the largest proportion of killed.

The writer's object in submitting this statement at this time is to point out certain sources of error which have in the past given to those concerned in selecting methods of disinfection a bewildering set of contradictory data to digest. Some of those to whom such contradictory results have been submitted have naturally enough become disgusted with all results of the same nature. Nor is it to be wondered at, when, for instance, one city adopts, on bacteriological evidence, five ounces of formaldehyde per 1,000 cubic feet, and another city, also on bacteriological evidence, has announced officially that over 70 ounces per 1,000 cubic feet is not uniformly efficient. The question will naturally arise: What character and conditions should a test organism have to yield a satisfactory and conclusive result? The answer is involved in what has been said already. Categorically, it should be fresh—not more than two days dried; and it should be really dry; while the species used should be those encountered in actual practice, or non-pathogenic forms, carefully tested and selected to show parallel resistance to those which it is desired to kill in practice. The reason for selecting dry organisms, dried, however, not more than two days, is simply that such organisms represent the conditions which those infective agents naturally distributed in the infected room will most probably present. Any organisms in the room freshly deposited by the patient just before death or removal, are likely to be moist, hence are likely to be killed readily. Those deposited by the patient a week or more before are likely to be dead, or at least dried to a point of low resistance, and are also readily killed. It is therefore the organisms that were thrown out by the patient from one to seven days before his death or removal that will usually prove the more resistant, and it is to these that the disinfector must pay attention. It is obvious that organisms of like age and dryness should be used for the tests. To kill organisms in this condition high humidity of the atmosphere is required if efficiency, with economy of gas, is desired.

In practice, bacteria dry more quickly on glass than on cotton or filter paper. This is probably the chief reason, if not the only one, why it has been noted by various observers that organisms dried on glass are more resistant (if not dried too long) than those dried on the other materials under like conditions. Hence glass objects for test organisms have, in the writer's hands, given the most generally reliable and uniform results.

METHODS OF BACTERIOLOGICAL EXAMINATION OF MILK.

FRANCIS H. SLACK,
Bacteriologist, Bureau of Milk Inspection, Boston Board of Health.

THE bacteriological examination of milk is rapidly becoming one of the essentials in the maintenance of public health. For years milk supplies have been inspected and tested to chemical standards alone, with the result that watered, adulterated, and preserved milks have been practically driven from the markets. These chemical tests are necessary, and only fail in that they do not go far enough, since a milk impure and unwholesome on account of bacterial growth, or a milk from a diseased animal, cannot be detected by them.

The bacterial count and the microscopical examination of the milk sediment reveal whether the milk has been properly handled or not, and, to a great extent, the condition of the animal from which it came.

It so happened that a conviction of the importance of this examination came to several cities at about the same time, and as no methods of milk examination had been agreed upon among bacteriologists, each laboratory started the work independently as best it might. As a result, a large number of laboratories make routine bacteriological examinations of milk, but cannot compare results, because of differences in methods.

The subject is of so much importance that these various methods of technique and differences of apparatus ought to be compared, in order that the best may be chosen; and definite, uniform methods for routine examination should be agreed upon.

It is with this object in view that the following technique and apparatus, developed at the Boston Board of Health Laboratory, under the direction of Dr. H. W. Hill, are offered for comparison and criticism.

Before milk work was begun, Dr. E. H. Wilson, of Brooklyn, courteously permitted Dr. Hill to examine and collate the replies from about 15 prominent laboratories doing milk work, made in response to a circular letter containing questions concerning technique, which Dr. Wilson had sent out. Although the replies showed wide differences in technique, even among workers devoted largely or solely to milk examinations, the technique in Boston was based on the consensus of the opinions given and modified from time to time as the work developed and became familiar to us.

For collecting samples, we have designed an apparatus using test tubes as containers; the samples, after thorough mixing, are transferred to the test tubes by means of large glass pipettes, a clean sterile pipette being used for each sample. The case for carrying the samples is made of copper, with double walls interlaid with half-inch felting, divided into three compartments, the central one for samples, the other two for ice. The test tubes are arranged in small racks, made of copper tubing weighted with a strip of lead and padded with rubber. Each rack holds four tubes. Holes in the bottoms of the partitions allow ice-water to circulate freely around the lower ends of the tubes. When this outfit is iced and closed, a constant temperature of 34° F. is maintained. The pipettes are carried beneath the sample case in a detachable copper box, adapted for sterilizing and divided into two compartments, the upper for clean sterile pipettes, the lower for the pipettes after use.

The use of the test tube has been adopted, in preference to the use of bottles, for the following reasons, dependent in most cases on the long slender shape of the tube:

1. Economy of floor area in the collecting case.

2. Avoidance of the necessity for two or more layers of containers, the lower layer of which would be always difficult of access.

3. The facility for maintaining low temperature by the circulation of ice-water about the lower ends of the tubes.

4. The ease with which all the usual washing, sterilizing, and general handling of test tubes can be done, since the test tube is a regular piece of ordinary apparatus, involving no departure from the ordinary routine in all the usual manipulations.

In plating, we have followed the methods recommended by the Bacteriological Committee.[1] We have found a dilution of 1:10,000 best suited for routine work. When examining samples recently taken from individual cows or from a milk supply known to be fairly pure, we dilute 100 times. When the microscopical examination, which will be described in detail later, shows a milk crowded with bacteria, we dilute 1:1,000,000. The lowest count we have recorded is 300 bacteria to 1 c.c.; the highest 640,000,000.

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[1] *Jour. Infect. Dis.*, Supplm. No. 1, 1905, p. 1.

For dilution water, square eight-ounce bottles have been found more easy to handle and more economical of space than other forms of bottles or flasks. Straight sided 1 c.c. pipettes are more easily handled than those with bulbs. They can easily be made from small glass tubing and calibrated in the laboratory.

For a medium, we use agar-agar made according to the directions of the Bacteriological Committee. A few changes have been made after careful comparison of results with varying acidities and percentages of agar. We have the best results with a 1 per cent agar, reaction + 1.5.

The additions of lactose or litmus to the medium has not, so far as we have tried it, proved of any special advantage.

We do not use gelatin, on account of the difficulty of maintaining uniform room temperature, and the length of time which must elapse before a report can be made.

The agar plates are incubated in a saturated atmosphere for 24 hours at 37° C.

Comparison of plates grown at room temperature with those grown at 37° C. has at times shown great differences in the number of colonies developing in duplicate plates. These differences, however, have not been uniform, the higher or lower temperature developing more colonies according to the nature of the organisms present. Incubation at 37° C. has been adopted, since it allows a much quicker report; it gives each sample the same treatment at a regulated temperature; and it allows a fair comparison of results obtained over long periods. If the plates are incubated 48 hours, a slightly higher average count will be obtained, not enough, however, materially to change the report. In many plates the count is lower at the end of 48 hours on account of small colonies becoming obscured in the growth of larger ones. There are also more spreaders, which means a greater loss of counts.

Porous Petri dish covers, suggested by Dr. Hill and since recommended by the Bacteriological Committee, have proved very efficient in reducing the number of spreaders. By their use in our work, spreaders have been reduced from 35 per cent to practically none.

Spreading under glass covers seems to be caused principally by water condensing on the inner surface of the cover and on the surface of the agar. The first fault can be partially overcome by inverting the dish, but the second seems to be unavoidable except by using the porous covers. With the glass covers, the bacteria in the surface colonies multiply rapidly in the film of condensation water, often spreading over the whole surface, and thus making a count impossible. The dry porous earthenware covers absorb this condensation water, still leaving the atmosphere saturated, as is proved by the quick growth and large size of the colonies. It is not necessary to invert the dishes; the covers are cheaper and more durable than glass, and they can be marked with lead-pencil, the marks erasing easily. Organisms having an inherent tendency to spread from unusual motility are not prevented from spreading by the use of these covers.

With the porous covers, as with the glass, each additional day's incubation shows an increase in the number of spreaders, due partly at least to the fact that the moisture gradually fills the interstices so that they do not absorb as readily as at first; but, day by day, the spreaders are much smaller in number than with glass covers.

It is quite essential to the best results that the porous covers should be washed as seldom as possible. In sterilizing them, the process should be prolonged over the time necessary to kill the organisms, in order that the covers may be thoroughly dry.

Our counting apparatus is simple and inexpensive. A circle, four and one-half inches in diameter, divided into 10 equal segments, is cut into the surface of a child's school slate; the lines are then filled with red lead, against which any colonies lying immediately over them, are easily seen. The surface of the slate, which tends to become gray, with time and use, may be kept black by occasionally rubbing with a little vaseline. The Petri dish is placed, uncovered, bottom down over the circle. A wooden box, six by six by five inches, with open bottom, glass front, and a four-inch circular opening in the top, the wooden parts painted black within and without to avoid refraction of the light, is placed over the plate and centered.

A common four-inch reading glass, magnifying about two diameters, fits over the opening in the top of the box, thus protecting the plate, keeping a constant focus and leaving both hands of the operator free. A tally record for adding and recording the counts by a simple pressure of the thumb for each colony seen, completes the outfit.

The advantages of this counting apparatus may be summarized as follows:

1. Cheapness.
2. The lens is held at the proper focus, leaving both hands of the operator free.
3. The whole field is exposed to view, so that there is no danger of counting the same colony twice.
4. The radial division into 10ths makes it easy in a crowded plate to obtain an approximate estimate by multiplication of the count of one or two representative sections.
5. The plate is so well protected from contamination that it may be counted face up with the cover removed.

For the microscopic examination of milk we first obtain the sediment from a known quantity by centrifugalizing; Stewart, of Philadelphia, uses an apparatus by means of which a large number of samples may be treated at the same time.

A modification of this consists of an aluminum disc and cover, the whole being 10 inches in diameter and $\frac{5}{8}$ of an inch in depth.

This disc is fitted to hold 20 small tubes arranged radially. The tubes hold about 2 c.c. each. Both ends are closed with rubber stoppers. By the use of these tubes, the whole sediment from a known quantity of milk is obtained, and may be spread over a given space. We have arbitrarily adopted a space of 4 sq. cm.

For smearing the sediment, slides nine by two inches of common window glass are convenient. One of these may be ruled with blue pencil into 11 spaces, each 2 by 2 cm., leaving a space 2 by $1\frac{1}{2}$ cm. at the top of each for the sample number and a similar space at the lower end for comments on the microscopical examination. By stopping the tubes at one end before opening the sample case, the time of exposure of the samples to room temperature is lessened. Each sample need be exposed only a few seconds while filling the tube, and immediately replaced in the case.

The milk sample is shaken 25 times, the tube is filled from the test tube by pouring, then stoppered, and finally inserted into its properly numbered receptacle in the disc. We centrifugalize 10 minutes at a speed of 2,000 to 3,000 revolutions a minute.

To obtain the sediment with least disturbance, first remove the stopper at the inner or cream end, then, holding the tube with the cream end downward, remove the cream with a platinum loop and pour the milk out; lastly, still holding the cream end down, carefully remove the other stopper with the adhering sediment and smear the sediment evenly, with a drop of sterile water, over the measured space on the glass slide, rubbing the stopper directly on the glass until all the sediment is removed. Dry with gentle heat and stain with methylene blue.

The examination of a properly prepared milk sediment under the microscope with a $\frac{1}{12}$ oil immersion lens gives a very good idea as to the number of bacteria present. We began this examination with the intention of looking for pus and streptococci alone. The variation in the number of bacteria in different samples was, however, so apparent under the microscope that it suggested this form of examination as a more convenient and quicker method than plating for determining the bacterial content of milk, or at least for eliminating samples comparatively free of bacteria.

We determined to test the question thoroughly by comparing the microscopical estimate with the actual count as obtained from the plates. The comparison was carried out very carefully with over 2,200 samples, each sample being subjected to the double test, i. e., plating and centrifugalizing; the microscopic estimate was made before the plate was counted and an error of less than 1 per cent was made in passing (as below 500,000 bacteria to 1 c.c.) milks which in the plates showed above this limit. Over a third of the total error occurred in the first 420 samples, before the method was fully developed.

This method would be useless in examining very clean milks for certification, as the lowest limit of accuracy would probably be around the 100,000 to 1 c.c. mark. Perhaps, by obtaining the sediment from a larger amount of milk, the test could be made more delicate.

If the microscopic estimate is made before the samples are plated, it is hardly necessary to plate those samples which are manifestly within the law's requirements. Besides such cases, many milks are obviously above the legal limit; these are plated in order to confirm the microscopic examination, and to have definite figures for legal purposes. Besides those entirely below and above, there are still others about which the examiner is in doubt. These of course need to be plated. Finally, of those apparently above the law, there are occasional samples which on plating run below it. It is still a question in such cases whether the plate or the microscope is the more correct. This group, although a very small one, makes the plating of high count milk more necessary, since it would be inadvisable for legal purposes to go into court on the microscopic estimate alone.

It is easy to determine, through the microscopic examination, what dilution will be necessary in plating, in order to ascertain correctly the number of bacteria present.

When examining the sediment for pus, since pus cells and dead leucocytes are identical, it is impossible to differentiate them under the microscope by the appearance of the individual cells. While it is not uncommon to find milks with these cells nearly absent, most milks have at least three or four such cells to the $\frac{1}{12}$ oil immersion field.

Opinions as to the number of such cells required to indicate pus differ. Professor Bergey, of the University of Pennsylvania,[1] says: "There is still no agreement among bacteriologists as to the number of cells in a specimen that will justify the diagnosis of the presence of pus. The number of cells in a field of the $\frac{1}{12}$ immersion lens is taken arbitrarily at 10. This number of cells per field may not always indicate pus, but it is believed that in the majority of instances it does indicate the presence of pus in milk derived from individual cows." Stewart, of Philadelphia, with the sediment from 1 c.c. of milk spread over a surface of 1 sq. cm., allows 23 cells to the $\frac{1}{12}$ immersion field before reporting pus. On account of the large amount of this milk with high cellular content at present in the markets, and the not wholly unreasonable doubt as to whether such milk is injurious to the public health, it has seemed fairest to all concerned, while fully recognizing that some abnormal condition must be present in the animal yielding such milk, to adopt a standard for market milk, for the present, allowing not over 50 cells to the $\frac{1}{12}$ immersion field (spreading the sediment from 2 c.c. of milk over 4 sq. cm.). So little of the sediment is seen at once with the high power that it is well to confirm the diagnosis of pus by making a thorough examination of the whole surface with a low-power lens, to determine how uniform a smear has been made. By using an eye-piece micrometer, ruled in squares, the relation of the area of one square to that of the $\frac{1}{12}$ immersion lens being previously calculated, a count may be made with the low-power lens.

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[1] *Bulletin 125*, Comwlth. of Penn., Dept. Agric., 1904.

Streptococci are, in our experience, seldom found to any great extent by direct microscopical examination. Occasionally a sample will be found crowded with long chains. More often streptococci, if present, are in the form of diplococci or very short chains. In any examination where streptococci, diplococci, or cocci are found in the sediment, and the plate from the same sample contains colonies resembling streptococci colonies in excess of a count of 100,000 to 1 c.c., we transplant these colonies to broth to see if chains will develop. We first make and record an estimate of the number of such colonies present, then transfer from 10 to 50 of them to broth, and grow for 24 hours at 37° C. Streptococci in small numbers are present in most market milk.

We condemn a milk for streptococci when these three tests are all positive:

1. Microscopic examination of the sediment showing streptococci, diplococci, or cocci.

2. The plate from the same sample showing colonies resembling streptococci colonies, in excess of 100,000 to 1 c.c.

3. The broth culture from these colonies showing streptococci alone or in great excess of the other bacteria present.

In conclusion, a word on the correlation of temperature and count. At first thought, one would always expect to find a high bacterial count in samples showing a high temperature, and a low count in low temperature samples, and if all the milk in question were of the same age and had been kept continuously at the temperature found, this would generally be the case. But it is easy to see that a high temperature might accompany a low count on milk only a few hours old which had not been properly cooled, while a low temperature and a high count are frequently found together in milk recently cooled which previously for 12 to 36 hours had been kept warm, as is often the case during transportation as practiced in some parts of the country. Thus, by taking temperature alone it is impossible to state whether or not a milk is good from the bacterial standpoint, and if milk is judged by the temperature standard alone, much that is good may be condemned, and very much that is poor will be kept on sale. Taking temperatures is important, and tells us how the milk is being cared for at time of sampling. By making bacterial counts, we find how the milk has been cared for up to the present, any lack of cleanliness or care being recorded by the corresponding increase in bacteria. By centrifugalizing and examining the sediment, we can estimate the bacteria present, and detect milk which on account of disease or dirt is unfit for use as food.

All of these methods of examination have been carefully worked out with checks and controls. Realizing that there is still great room for improvement in many ways, we recommend them as a system which has been reasonably successful in practice, for city laboratory work where quick reports are needed as well as the accurate examination of large numbers of samples. We do not offer them as developed to a final point of simplicity and efficiency; our hope is only that their presentation here may serve to provoke discussion and to secure ultimate agreement on uniform and efficient methods.

SUGGESTIONS FOR CHANGES IN THE SCHEDULES FOR MAKING BROTH, GELATIN, AND AGAR, RECOMMENDED IN THE LAST REPORT OF THE COMMITTEE ON STANDARD METHODS OF WATER ANALYSIS.[1]

Hibbert Winslow Hill,
Minnesota State Board of Health Laboratories, Minneapolis, Minn.

Suggestion 1.—In preparing broth, gelatin, and agar, transfer the direction to "adjust to the required reaction," from its present position to precede immediately the direction for heating over the water bath for half an hour.

The reason for this change is that, in order to secure uniformity in successive lots of media, it is well that *all* heating should be done at a uniform reaction. If the recommendations as they stand now are followed, the half-hour's heating on the water bath is given before the adjustment to a fixed reaction, i. e., at the casual reaction which the meat infusion may happen to have, further modified somewhat by the addition of peptone in all cases, and, in the case of gelatin, very much modified by the presence of gelatin. If, however, the change here suggested be adopted, the reaction at which all heating is done will be the same throughout for all media *which are to have the same final reaction.* It will be remembered that the earlier recommendations of this Committee called for neutralization before heating, and this provided that the reaction at which all heating was done should be the same throughout for all media, *without regard to the final reaction.* My suggestion, if adopted, will return as closely as may be to uniformity under the modified conditions as regards the final reaction now generally adopted, i. e., the adjustment to a final reaction without previous neutralization.

Suggestion 2.—In preparing broth, gelatin, and agar, make the period of boiling over the free flame five minutes instead of two minutes as in the present recommendation. This also is a return to the recommendations formerly made.

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[1] *Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 1.

The reason for this change is, that it seems to be a fairly common experience that two minutes' boiling is hardly sufficient to precipitate thoroughly the albumens present at the reaction at which the boiling is done. Five minutes' boiling, on the other hand, is usually sufficient for precipitation, and obviates subsequent precipitation during sterilization.

Suggestion 3.—Since, in the adjustment of agar media by titration, some little difficulty is encountered at times, if it be done after the addition of the 3 per cent agar, and especially if the titration process be prolonged, because of the agar becoming cold, it is at times convenient to adjust the reaction of the meat infusion plus peptone before adding the agar. If this be done, the reaction to which the meat infusion is adjusted should be double that which is desired as the final reaction. The subsequent addition of the agar in 3 per cent strength, as recommended, brings the reaction, as well as the percentage of peptone and of meat infusion constituents, to the proper point. Since it is difficult to see that the adjustment of the agar reaction before or after the addition of agar can make any difference in the composition of the medium, it would seem that an alternative method might here be provided without infringing on the principle of securing strict uniformity. The 3 per cent agar being neutral, exactly the same amount of alkali is necessary to secure the same final reaction from either the meat infusion plus peptone, (double strength) brought to double the final reaction, and then diluted one-half by the 3 per cent agar or from the whole agar medium (final strength) brought directly to the final reaction.

Summed up these suggestions would be as follows:

1. In the present form of recommendations for gelatin and agar, p. 108 of Supplement No. 1 of the *Journal of Infectious Diseases*, strike out steps 11 and 12; and insert them again, renumbered 9 and 10, respectively; strike out 9 and 10 as they now are, and reinsert them, renumbering them 11 and 12, respectively.

2. In step 13, strike out "two minutes" and substitute "five minutes."

3. Insert a footnote, referring to steps 8, 9, 10, as renumbered in accordance with the above suggestions, to read:

If preferred, meat infusion and peptone of double strength, intended for the preparation of agar media, may be adjusted to twice the final reaction desired, before the addition of the 3 per cent agar. The addition of the agar should then be made between steps 10 and 11 (as renumbered).

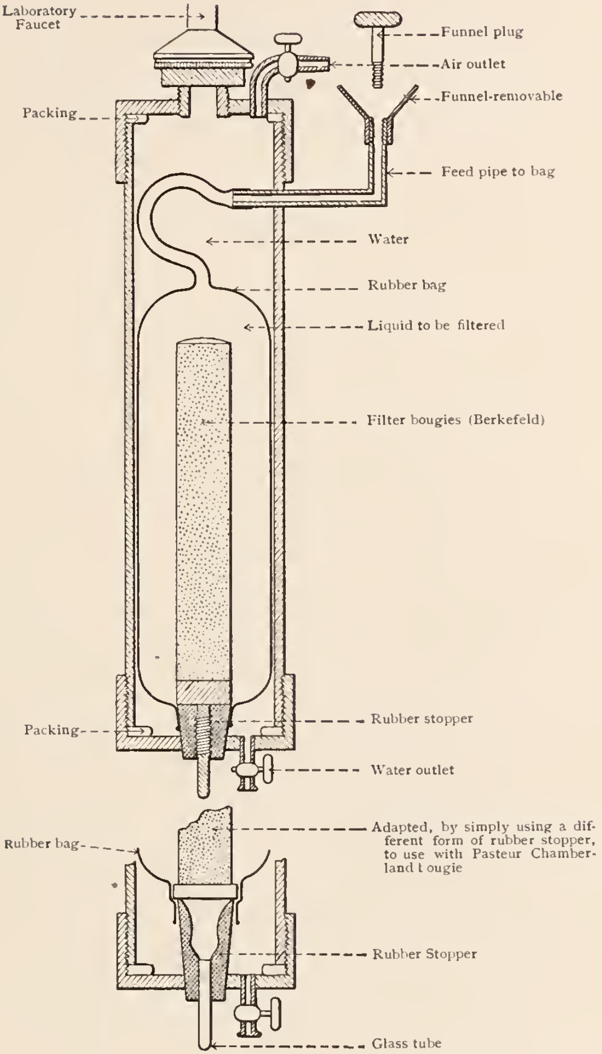
A DEVICE FOR FILTERING TOXINS, ETC., BY THE USE OF WATER PRESSURE.

HIBBERT WINSLOW HILL.
Minnesota State Board of Health Laboratories, Minneapolis, Minn.

THE use of gas or air pressure to drive toxins, or other bacterial fluids, which it is desired to filter, and at the same time to sterilize through bougies of porcelain etc., has been common for a long time. The ordinary aspiration filter is, of course, really a pressure filter, the limit of pressure being rather less than one atmosphere, and depending on the degree of exhaustion obtainable. But direct air or gas pressure—secured say by compression—has always presented, besides the mechanical difficulties, chemical or physical difficulties, dependent on the pressure-modifications of the action of the gases used upon the liquids to be filtered.

It occurred to the writer that the interposition of a thin sheet of rubber between the surface of the liquid to be filtered and the liquid (air, gas, or water) which was used as the vehicle to transmit the pressure would at once remove almost all the objections which could be brought against pressure filtration. Because of its relative density and inelasticity, water presents so many mechanical advantages over air, that attention was concentrated wholly upon the use of water pressure.

The device illustrated by the accompanying drawing is extremely simple in principle. A strong metal cylinder of appropriate size to contain the bougie, and a rubber bag, made to surround the bougie and to contain, say from one-half to one liter of the liquid to be filtered, are the sole requisites besides sufficient water pressure. Water may often be obtained from the laboratory tap under pressure of from 45 to 90 lbs.—say from 3 to 6 atmospheres. A small force pump may readily be provided which will supply 200 to 300 lbs. pressure. This water is admitted to the cylinder upon the outside of the rubber bag containing the liquid to be filtered. The pressure drives the rubber inward upon the contained liquid, and so upon the contained bougie, with a perfectly adjusted and uniformly dis-



Adapted, by simply using a different form of rubber stopper, to use with Pasteur Chamberland bougie tributed pressure. There is nothing to tear the rubber, which may be very thin; every drop of the contained liquid is filtered; the filtration is very rapid; and there is no question introduced as to the effect of the exposure of the liquid to air or gas under pressure, for it has had no such exposure. To reduce the pressure from say 200 lbs. to normal, it is only necessary to shut off the source of the water pressure by turning a tap, and to allow the escape of a few cubic centimeters of water from the cylinder. The rubber bag may be refilled after each emptying very readily.

THE FERMENTATION TUBE IN THE STUDY OF ANAEROBIC BACTERIA WITH SPECIAL REFERENCE TO GAS PRODUCTION AND THE USE OF MILK AS A CULTURE MEDIUM.*

THEOBALD SMITH, HERBERT R. BROWN, AND ERNEST L. WALKER.
(From the Laboratory of Comparative Pathology, Harvard Medical School.)

THERE has been going on in recent years considerable activity in the study of anaerobic bacteria. Hitherto more or less neglected, this large group is being recognized, and its relation to the processes of putrefaction and to various forms of disease subjected to more rigid scrutiny.

The difficulties which surround the prompt recognition of the presence of anaerobes and their isolation and identification are in the main responsible for our inadequate knowledge of their activities, benign and malignant. That these difficulties are being appreciated is made evident by the large number of publications devoted chiefly to the technics of cultivation which have appeared during the past 10 years. Not a few species have been described at the same time, but there is evidence on all sides that these descriptions are inadequate. Let anyone endeavor to identify a newly isolated form with those already described, and the uncertainty of the species diagnosis appears at once to confound the reader. The temptation to create new species adds to the confusion.

In order to place the study of anaerobes on a better basis, it is obviously necessary to bring out as many different morphological and physiological characters as possible, and to make the determination of these characters as easy and simple as is compatible with accuracy. No one test can be relied upon definitely to circumscribe any species; this must be hedged in by as many different tests as can be devised. In fact every new species studied may add to the number of tests already in existence by revealing some unexpected variation.

In the following pages we wish to call attention to certain uses of the fermentation tube which tend to lessen materially the labor involved in the study of morphological and physiological characters.

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*Read before the laboratory section of the American Public Health Association, September 25, 1905

Since 1890 one of us has repeatedly called attention to the fact that anaerobic bacteria which refuse to multiply in ordinary culture tubes do so readily in the fermentation tube.[1] It was also pointed out, at that time and subsequently, that in the closed arm of the fermentation tube reducing processes are at work which slowly use up any free, available oxygen, and thus maintain a condition favorable to anaerobic bacteria. If ordinary sterile broth in such a tube be tinted with methylene blue or litmus, the coloring agent becomes reduced, and the closed arm remains colorless for a long period of time.[2]

These publications did not, however, lead to any extended use of the fermentation tube in the routine study of anaerobic bacteria. The main reason for this neglect is the fact that this tube has been regarded solely as a means of studying gas production, and its great value as a general culture tube has been overlooked. Another reason for its neglect is, perhaps, that nearly all anaerobes produce gas, and hence, it might be inferred, cannot be differentiated with the aid of any method which reveals gas production. Another reason which, though not expressed, can be read between the lines in some publications, seems to presuppose that anaerobic bacteria must be cultivated in media completely deprived of oxygen although we have no certain knowledge that in nature they actually do multiply under such conditions.

The essential problem in the study of anaerobes is not so much the exclusion of oxygen as the supply of special kinds of assimilable foods. These seem to vary very much, according to the species or group of species under observation. In addition to the albumoses and sugars of our ordinary broth, the proteid substances of animal tissues and fluids are especially favorable to many species. It might therefore be taken for granted that to establish simply anaerobic conditions is not always sufficient. It was noticed that anaerobes do not always multiply in the fermentation tube containing the ordinary peptone broth. A step in advance was made by adding bits of sterile tissues of animals; to this, attention was called by one of us many years ago.[1] We also find that Kitt describes multiplication of certain anaerobes around bits of muscle tissue.[4] In 1899 special attention was called to this method as one which never failed to induce multiplication.[3] Even when sugars have been removed by fermentation, or when the culture tubes have remained unused for weeks and have partly dried out, multiplication takes place in the presence of sterile tissue, provided the evaporated fluid is replaced by sterile water.

The use of bits of sterile tissue to stimulate the multiplication of anaerobes in media exposed to the air has been recently rediscovered by Tarozzi.[5] He is equally satisfied as to its efficacy in favoring the growth of anaerobes, when oxygen is not rigidly excluded.

Provided with this means, one of us began, in 1900, to study more systematically the gas production of anaerobic bacteria isolated from various sources. Since that date the method has been repeatedly tried by us on the same bacteria, kept under cultivation in deep layers of agar to see whether the type of gas production would change under prolonged cultivation. It is upon the results of this special study that this paper is based.

No attempts will be made at present to identify accurately the anaerobes isolated by one of us with species already named. Our object at present is simply to exhibit the workings of the method, and to add one more diagnostic procedure to those now in use in the study of anaerobes.

The cultures which we have used are designated as follows:

Rauschbrand, Mass.—Culture isolated from a cow in August, 1900, and kept growing since then in tubes of deep agar. This bacillus was promptly fatal to mice, guinea-pigs, and rabbits after injection into the subcutis. The lesions consisted in a sanguinolent edema in the subcutis at the seat of inoculation, with or without gas bubbles.

Rauschbrand A_1.—Received from a manufacturer of Rauschbrand vaccine, August 1900.

Rauschbrand A_2.—Received from the same source in 1904.

Rauschbrand A_3.—The same culture passed through a cow before it was received.

Rauschbrand B.—Received from another manufacturer in 1900.

The two following cultures are provisionally named "malignant edema," although this classification may need revision.

B. malignant edema I.—Isolated in 1897 from a guinea-pig inoculated with tuberculous tissue from cattle. The tissue had probably been contaminated in the abattoir.

B. malignant edema II.—Isolated in 1899 from an inflammatory swelling on the leg of a cow. Markedly virulent for guinea-pigs.

Anaerobe VIII.—Isolated from fermented broth, not completely sterilized, in 1897.

Anaerobe XI.—Isolated from a similar source in 1898.

Anaerobe XV.—Isolated from the organs of a pig in an outbreak of hog cholera in May, 1901.

Anaerobe XVI.—Isolated from a similar outbreak in May, 1902.

B. aerogenes capsulatus I.—Received from Professor E. O. Jordan, University of Chicago, in March, 1904.

B. aerogenes capsulatus II.—Received from Dr. W. W. Ford, Johns Hopkins University, December, 1904.

I. MULTIPLICATION AND SPORE FORMATION.

In bacteriology cultures serve two main purposes: to maintain the life of microorganisms indefinitely, thereby making them accessible to morphological and physiological studies; and secondly, to reveal differences of form and function which may be used for identification and classification.

The fermentation tube, containing ordinary or even fermented broth plus the sterile tissue of some animal, admirably serves the purpose of inducing multiplication and spore-formation. The bacteria are readily accessible at all times, and may be removed with a pipette drawn out into a capillary extremity and provided with a rubber nipple.

To prepare the tubes, we have used guinea-pigs and rabbits. These animals are chloroformed, not killed by a blow, as this procedure may cause rupture of vessels and the introduction of bacteria from the surface of mucous membranes. The animal is thoroughly moistened with water, the skin reflected and then the abdomen opened with sterile instruments. Sterilized forceps, held in readiness, are used to remove from the liver, spleen, or kidneys bits of tissue just large enough to pass into the narrow part of the fermentation tube. The tissue is pushed into this narrow portion, but not necessarily beyond. After incubation for two or more days the tubes are ready for use. From 10 to 20 tubes may be prepared from a single guinea-pig.

We are well aware that the organs of animals are not always sterile,[6] but the number of tubes prepared in this way which have subsequently contained living bacteria, when young, vigorous adults are used, is so small that no apprehension need be felt on this account. The tubes are inoculated by transferring bits of agar, broth, or tissue containing bacteria, to the bulb. It is not necessary to force the transferred material into the branch. With anaerobes, the promptness with which multiplication takes place often depends upon the amount of culture material transferred. Hence we have been in the habit of transferring a drop or two drawn from the bottom of the bulb with a pipette rather than a loop, since the largest number of bacteria and spores will be found in the connecting tube. Cultures may be maintained in this way indefinitely, for the study of morphological details, without resorting to deep agar or gelatin, or the use of alkaline pyrogallol in one way or another.

2. SELECTIVE ACTION UPON CARBOHYDRATES.

In exploiting the gas production of facultative anaerobes for diagnostic purposes, two different manifestations of fermentative activity may be noted: first, the selective action on different carbohydrates and the production therefrom of acids and gases, or acids only; and second, the gas formula $\frac{H}{CO_2}$ as roughly determined by the method used by one of us in 1890.[4]

Among facultative anaerobes, gas production depends exclusively upon the presence of some fermentescible sugar. When this is absent, there is not only no gas production, but the bacterium is unable to multiply under anaerobic conditions. Hence the freedom from cloudiness of the closed arm of the fermentation tube when the appropriate carbohydrates are absent.

Among anaerobes the conditions are nearly the same, in that large amounts of gas are always associated with the presence of some sugar; but even in tubes which, according to tests with B. coli, are free from sugar, some anaerobes may multiply and produce a little gas.* Evidently some of the proteid matter may yield gas under the fermentative activity of anaerobes. When bits of sterile tissues are introduced into fermented broth, many anaerobes produce a considerable amount of gas. Bits of tissue as large as a small bean may give rise to as much as 5 to 8 c.c. of gas.

In studying the selective action of anaerobes upon different carbohydrates, it is therefore desirable to induce multiplication in fermentation tubes containing fermented broth and sugars without tissue, whenever possible. We have succeeded quite uniformly by the following simple method. Fermentation tubes containing fermented broth, not over three or four weeks old and duly sterilized, receive 1 per cent of some sugar from a sterile 10 or 20 per cent solution, with a suitable sterile pipette. The tubes are then steamed in the Arnold sterilizer. This thoroughly mixes the sugar with the broth, and also puts the broth into the condition most favorable for the anaerobes. After adding the bacilli to the fluid in the bulb, it is well to shake gently, then allow a little broth to run out of the branch and back again, to insure the presence of bacteria in the closed arm. Our observations have been extended to three sugars only—dextrose, saccharose, and lactose.

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* This is true of Anaerobe XV, which multiplies and produces spores in fermented peptone broth n the fermentation tube.

In the dextrose tubes there was rapid multiplication with evolution of gas. From 40 to 100 per cent had accumulated in two or three days.[*] In the tubes containing the other sugars there was also, with two exceptions, active multiplication, with variable gas production depending on the species under observation. B. aerogenes capsulatus produced gas in abundance in the three sugars. Rauschbrand, Mass., and Anaerobe XVI produced over 80 per cent in lactose, as well as in dextrose broth. In the other tubes, amounts of gas from traces to 5 per cent only accumulated. It is obvious that,

DESIGNATION OF CULTURE	IN FERMENTED BROTH, WITHOUT TISSUE, PLUS 1 PER CENT OF			GAS FORMULA[†] $\frac{H}{CO_2}$
	Dextrose	Saccharose	Lactose	
Rauschbrand, Mass	+	−	+	$\frac{5}{3}$ to $\frac{3}{1}$
" B	+	−	+	$\frac{5}{3}$ to $\frac{2}{1}$
" A_2, A_3	+	−	−	$\frac{1}{3}$ to $\frac{2}{5}$
B. malignant edema I	+	−	−	$\frac{1}{3}$ to $\frac{2}{5}$
Anaerobe VIII	+	−	−	$\frac{1}{4}$ to $\frac{1}{6}$
" XI	+	−	−	$\frac{1}{3}$ to $\frac{1}{4}$
B. malignant edema II	+	+	+	$\frac{2}{1}$ to $\frac{1}{2}$
Anaerobe XV	+	−	−	$\frac{4}{3}$
" XVI	+	−	+	$\frac{2}{1}$ to $\frac{5}{3}$
B. aerogenes capsulatus I, II	+	+	+	$\frac{3}{1}$ to $\frac{3}{2}$

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[*] See next section for an explanation of these figures. These fractions are approximate only, and give the variation with different amounts of gas produced, at widely different times, i. e., within a period of one to five years. The best results are obtained by stimulating to maximum gas production by repeated transfers, fresh media, etc. In but one culture (B. malignant edema II) is the gas formula too variable to be of any use. It is barely possible that this may be a mixture of two anaerobes, although we have not been able to substantiate this hypothesis as yet.

[†] Certain bacteria, among them the various races of tetanus bacilli and the B. necrophorous, produce only a little gas, even when sterile tissues are present.

to obtain a true selective action of the anaerobes, the broth should not contain more than traces of muscle-sugar, for this is acted upon as dextrose is. We recommend the removal of the muscle-sugar, as described by one of us.[1] In case fermented broth is not at hand, controls without any sugar should be made.

The preceding table is constructed from several experiments made to determine how far the selective action of the different cultures may be regarded as constant. The sign + indicates from 25 to 100 per cent gas, accumulating after two or more days. The sign − indicates from a bubble to 5 per cent of gas. Traces of gas are almost always produced, possibly from the peptones present.

Under certain conditions, as yet unexplained, the culture tube may remain absolutely clear, though under the same conditions at other times vigorous multiplication and gas formation goes on. Under such conditions a second inoculation is advisable. We have found that transfers from old cultures may fail, possibly because there are only spores left. Hence it is recommended to freshen the cultures by inoculating into one or two tubes in succession before making any important comparative tests.

3. THE GAS FORMULA OF ANAEROBES.

Special attention was given to the quantitative relation of CO_2 (as indicated by the amount of gas absorbed with KHO) and the explosive residue, which, for convenience, we will call hydrogen.

Since 1900 the various cultures on hand have been subjected to repeated tests to determine if the gas formula remained constant under cultivation. These repeated trials have shown that, with but one exception, the gas formula not only remains the same, but that it does not matter whether animal tissue be present or absent. Furthermore, the formula is the same in tubes containing different sugars. The exception noted is the culture provisionally called B. malignant edema II.

Three different types of fermentation have presented themselves to us:

1. The type in which the hydrogen predominates and in which the fraction $\frac{H}{CO_2} = \frac{4}{3}$ to $\frac{3}{1}$.

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[1] *Jour. Exper. Medicine*, 1899, 4, p. 375.

2. The type in which the carbon dioxide predominates and in which the fraction $\frac{H}{CO_2} = \frac{1}{6}$ to $\frac{2}{5}$.

3. An intermediate type, in which the fraction $\frac{H}{CO_2}$ is sometimes more, sometimes less, than unity.

To Group 1 belong Rauschbrand, Mass., A, and B, Anaerobes XV and XVI, and B. aerogenes capsulatus.

To Group 2 belong Rauschbrand A_2 and A_3, Anaerobes VIII and XI, and B. malignant edema I.

To Group 3 belongs B. malignant edema II.

Under these groups it is possible to establish subgroups according to the value of the fraction $\frac{H}{CO_2}$.

We content ourselves with this brief synopsis of the gas formula, which represents the summary of many hundred tests, and leave the details until each species has been more thoroughly studied with reference to other cultural characters.

4. MILK AS A CULTURE MEDIUM FOR ANAEROBES IN FERMENTATION TUBES.

The behavior of anaerobes in milk is occasionally mentioned in bacteriological literature, but such mention is meager and unsatisfactory. We have recently given special attention to this culture medium, and have found such a variety of changes, recognizable by the simplest means when fermentation tubes are used, that we recommend it most urgently as a routine test. We have not as yet studied the changes which occur in milk in any detail, and at present we simply point out the most obvious phenomena.

The preparation of the culture medium requires some care. The difficulty of removing all bacteria by discontinuous steaming is very great, and errors may creep in unless control observations are made. During the past summer we have found that steaming milk three times on consecutive days is quite insufficient to sterilize it. In one lot of tubes, only one out of 12 remained unchanged. In another lot, steamed four times, only one-half were actually sterilized. This inefficiency of discontinuous steaming may be in part due to the foaming of the milk and the consequent wetting of the cotton plug, where spores may lodge and fail to germinate. But this factor is more apparent than real. The majority of the tubes break down under the influence of spore-bearing anaerobes, which would not come into play in ordinary test tubes, where they would lie dormant, at least while the tube remained unused.

It may thus be said that if milk in fermentation tubes remains unchanged it is truly sterile, being free from anaerobes as well as aerobes.

In order to determine the most reliable procedure for sterilizing milk without resorting to the autoclave, various experiments were made. The following procedure was found effective. The milk is steamed in an Arnold sterilizer four times. Between the second and third steamings the tubes are placed in the incubator over night, or about 16 hours. Between the third and fourth steamings, one or two days may intervene, the tubes being kept in the incubator 24 hours. After the process is completed, fresh cotton wool plugs from sterile test tubes are used to replace the old ones. At least a week should elapse before the tubes are inoculated. During this time they should remain in the incubator three or four days. It is not improbable that autoclaved milk may still serve the purpose of differentiating anaerobes as satisfactorily as discontinuous steaming. Experiments are now under way which will serve to compare the action of the same anaerobe upon milk sterilized both ways. It is furthermore probable that the difficulties encountered in summer with this highly infected fluid may be very much lessened during the colder seasons of the year.

As regards the kind of tubes to be used, some suggestions may not be amiss. It may seem to some a hopeless task to clean fermentation tubes filled with coagulated milk, yet the difficulties are not so great as anticipated. If the milk is clotted, steaming or autoclaving will force much of the clot out, and open the tube for the subsequent use of some alkali, such as carbonate of soda or caustic soda or potash, to dissolve the coagulum under the influence of the heat. Lastly, the tubes may be filled with ordinary cleaning mixture (sulphuric acid and bichromate of potash) and set aside for a few days.

The ordinary fermentation tube may be replaced by the tube as modified by H. W. Hill.[7] In these tubes the closed branch is provided with a removable ground glass cap. We have used the precaution to cover this joint with tin foil, and keep it there during sterilization and incubation. This protects the joint from contamination. It is highly probable that these tubes can be further simplified and cheapened by using an ordinary rubber stopper in place of the ground glass stopper, and protecting with tin foil. These tubes are much more easily cleaned than the ordinary form, and they also permit access to the closed branch without disturbing the fluid in the bulb, if a cork or rubber stopper be previously forced into the mouth of the bulb. There is, however, one precaution to be observed in the use of this modification. I have already referred to the possibility of contamination through the stopper in the warm, saturated atmosphere of the incubator. There is danger that the stopper may be forced loose when gas collects over the clotted milk and the clot is being forced out under pressure. Air may then be admitted, and give a fictitious value to the gas, both as to amount and formula.

In a number of publications E. Klein[8] has called attention to the presence of anaerobes in milk, more particularly of one named by him B. enteritidis sporogenes and regarded by him as the cause of certain epidemics of diarrhea. His method of isolating this organism is to heat milk to 80° C. for 15 minutes, and then incubate it in Buchner tubes, using alkaline pyrogallol to remove the oxygen. This method can of course be regarded only as a preliminary for the subsequent isolation by plates, for we have encountered several anaerobes in the same tube after three steamings. To isolate spore-bearing anaerobes from milk, we would recommend the simpler device of using the fermentation tube as modified by Hill, which permits us to avoid the bulb, where spore-bearing aerobes may be multiplying. This so-called "Anreicherungs verfahren" can be further utilized by inoculating small amounts of the treated milk into a series of fermentation tubes containing broth, as a substitute for plate cultures.

In fermentation tubes containing milk we have had no difficulty in obtaining active multiplication of all the anaerobes tested. The characters of the milk cultures examined differ widely for different species, but are remarkably uniform for the same species. Among them the most important arise from modifications of the following phenomena:

(1) Coagulation of milk, and the time required; (2) subsequent

liquefaction of the clot, and the time required; (3) gas production, slow or rapid, and gas formula; (4) production of acid; (5) odor.

The variety of cultural characters is best shown by classifying the anaerobes we have studied according to their behavior in milk. Four groups may be sharply defined and under each subdivisions may be made according to the rapidity of the process:

1. Bacilli producing coagulation after a variable number of days. Rauschbrand, Mass., and B. anaerobe XVI.

2. Bacilli coagulating milk and producing gas; (*a*) up to 50 per cent, Anaerobe XV; (*b*) up to 100 per cent, B. malignant edema II.

3. Bacilli causing slow or rapid digestion of the casein with slow production of gas up to 50 per cent. Odor very offensive. Acidity, 4 to 6 per cent. B. malignant edema I, Rauschbrand A_2, A_3, and Anaerobes VIII and XI.

4. Bacilli producing very rapidly a large amount of gas. Formula $\frac{H}{CO_2} = \frac{2}{1}$ to $\frac{4}{1}$. Milk coagulated and probably partially digested. Odor not offensive, sour. Acidity, 4 to 5 per cent. B. aerogenes capsulatus I, II.

The close parallelism between gas formula and behavior in milk of the cultures marked Rauschbrand A_2, A_3, B. malignant edema I, Anaerobes VIII, and XI makes it highly probable that they belong to the same species. The morphology supports this view, but further studies are necessary to decide this point.

5. WHAT IS RAUSCHBRAND?

Among the pathogenic species of anaerobic bacteria there are two which have been recognized as such for many years. These are the bacilli which produce malignant edema and *Rauschbrand* or blackleg. Throughout bacteriological literature there is an uncertainty as to the distinctions to be made between the agents of these two affections.

During the course of these studies we have encountered two wholly different types of Rauschbrand bacilli, both as regards gas formula and behavior in milk. They belong, in fact, to different species. In the case of one manufacturer of vaccine, the culture of 1904 belonged to one group, that of 1900 to the other. The latter agreed in its gas formula with the culture isolated by one of us as Rauschbrand, and with Rauschbrand B. It becomes a question of considerable importance whether Rauschbrand A_2 and A_3 can protect against a disease produced by bacilli of the other group, and whether to the first or to the second group should be accorded the right to bear the name of the disease in question.

We have been able to convince ourselves that the test upon small animals does not help us much in this dilemma. In fact this is somewhat misleading, since both types of bacilli may be fatal to guinea-pigs and produce somewhat similar lesions. If cultures A_2 and A_3 are genuine Rauschbrand bacilli, it would be impossible to exclude anaerobe VIII and B. malignant edema I. This would mean that this bacillus is very widely disseminated. But the preponderance of evidence is in favor of the other group as being the true Rauschbrand bacilli.

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SOURCE AND SIGNIFICANCE OF NITRITES IN STREAMS.

ELLEN H. RICHARDS.

THE history of water analysis shows from the first a difference of opinion, or at least a doubt, in the minds of chemists as to the source of nitrites, and as to the weight to be given to their presence as an indication of definite contamination.

The prevailing belief has probably been that expressed in the following statement of a distinguished chemist, made in 1902: "Nitrites are oxidation products derived from the nitrogenous organic matters above referred to. In the conversion of objectionable and harmful nitrogenous organic matters into innocuous substances, the nitrites mark a stage following that which is indicated by the presence of free ammonia."

While this may be true in some laboratory experiments the conditions must be carefully scrutinized before a broad generalization is safe. Experiments in the author's laboratory have shown it to be practically universal that solutions containing urine do develop nitrites, apparently from the ammoniacal compounds.

Sewage mixtures and sewage polluted streams show nitrites in a a greater or less degree, coexistent with lessening amounts of ammonia. Is it safe to assume that in all these cases the nitrites are the second stage in the cycle of nitrogen, toward nitrates from ammonia? It has been recognized for a long time that in wells, at least, nitrites signify a reduction of nitrates previously formed, and that this reduction is often effected by harmless means. We no longer condemn a well water on the evidence of nitrites alone, the simultaneous presence of ammonia is needed. But just what does that signify? Do we ever find high nitrites without previous nitrates?

The complete reduction under laboratory conditions of as much as 40 parts per million of nitrates to the same amount of nitrogen as nitrites by the action of the fermentation set up in the solution of city water to which has been added, 1 c.c. per liter of unsterilized milk and the complete disappearance of the nitrites before the organic nitrogenous matter went over into ammonia and without the presence of either sewage or, presumably, of pathogenic germs, inclines us to hesitate. Is it possible that the yeasts and lactic ferments always present in town sewage may be a factor in the production of nitrites and that the latter may not be so deadly as we once thought?

It is probable that many, if not most, kinds of bacteria reduce nitrates under favorable conditions, wherein then lies the significance of nitrites?

We have generally held that whatever the cause, the simultaneous presence of ammonia and nitrites in a water constituted a sufficient indictment, but are we justified in assuming pollution of streams whenever nitrites are present above three or five thousandths? How is the analyst to know whether the offending substance is derived from oxidation of ammoniacal compounds, presumably accompanied by a pathogenic organism, or from reduction of nitrates by fermentation? Is there not a vast difference in the significance of the results in the two cases? Again, is the quantity of nitrites found any indication of the amount of pollution?

These have been perennial questions in our laboratory but the subject came up in renewed importance in connection with the interpretation of the results of the Chicago Drainage Canal investigations. Did the presence of an excessive nitrite in the St. Louis intake mean a stage in the purification of the water of the canal, or was it only due to reduction of nitrate by the largely vegetable organic matter of the Missouri?

Did the variation in amount at the various points mean, as one of the chemists wrote: "it becomes evident that the increase in the proportion of nitrites becomes marked very much nearer the source of the sewage," or does it mean that whenever a stream brought in a large proportion of *nitrates* then a larger amount of nitrogen as nitrites appeared, because nitrates were decomposed?

That the latter was at least a predominant cause is indicated in the fact noted, but not explained, in the report, that "the point at which the maximum quantity of nitrates is found is at Ottawa. It is notable that the minimum is less, and the maximum never reaches the high proportion found" the year before the canal was opened.

This is difficult of explanation on the current theory but it is plain if the reduction theory is true. Since for some reason, low water perhaps, the Kankakee River, which enters above, and always carries high nitrates, in 1899 contained a decidedly larger amount than in 1900. Therefore evidences of greater reduction might be expected. Also the Desplaines River showed lower nitrites, although higher ammonia after the flow was increased by the canal.

Another instructive case has come under our hand. That of water carrying high nitrates mingling with polluted water and as a result a disappearance of nitrate and great increase of nitrite.

In this case there is no doubt in my own mind as to what happened. The reduction of nitrate takes place much more quickly than the oxidation of ammonia. Variations in the per cent of dissolved oxygen will affect the results more than any other condition after that of the presence of fermenting substances. Nitrites are an exceedingly unstable form of nitrogen, and conclusions drawn from observations at considerable length of time apart may be misleading.

THE USE OF HISTOLOGICAL METHODS IN THE RAPID AND CERTAIN DIAGNOSIS OF SMALLPOX.

WILLIAM TRAVIS HOWARD, JR.

IN April last, the author published a paper,[1] advocating the use of excised pieces of skin, fixed in Zenker's fluid, and sectioned in paraffin, in the diagnosis of doubtful cases of smallpox. The diagnosis is based upon the characteristic changes met with in the various stages of the smallpox lesion, and upon the presence of Cytoryctes variolae therein. In the one case on which the author had the opportunity of testing the method, good sections were had and a positive diagnosis was made within less than 60 hours after the material was obtained.

Two weeks after the author's paper, Thompson[2] proposed using, for the same purpose, the new acetone method of Henke and Zeller for fixation and sectioning. Thompson, who tested his method with success on several cases, obtained good sections in three hours after excision of the skin.

The author's object in the present paper is to call attention to this method and to urge its use for the rapid and certain diagnosis of clinically doubtful cases of smallpox, with the view of stamping out epidemics in their incipiency.

For the details of the methods and for the particulars in regard to the appearances upon which the diagnosis of smallpox is based, the reader is referred to the papers of Thompson and Howard.

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[1] *Jour. Amer. Med Assoc.*, April 8, 1905.
[2] *Ibid.*, April 22. 1905.

A FIELD KIT FOR PRELIMINARY WATER SURVEY.

Ellen H. Richards,
Massachusetts Institute of Technology, Boston.

Paper published *Technology Quarterly*, 1904, 17, pp. 277–80.

THE NEED FOR UNIFORM METHODS IN THE SANITARY EXAMINATION OF MILK.

S. C. PRESCOTT.

IN the older and larger cities, at least, the problem of a satisfactory milk supply has increased in complexity and seriousness as cities and urban communities have increased in population and have encroached upon the regions formerly devoted to agricultural pursuits. Thus the sources of milk production have been pushed farther and farther from the centers where the milk is consumed, and the business of supplying the inhabitants of a city with milk has become one of greater complexity. The water for a city is, in general, derived from a single large source, or few sources, on areas which may be so safeguarded as to prevent pollution with pathogenic or intestinal bacteria. Milk, on the other hand, is obtained from a large number of small sources, and is constantly subject to contamination because of the methods of handling, from the time it is drawn from the cow to the time it reaches the consumer. That milk is a vehicle for the ready transmission of the bacteria of infectious diseases is evident from the large number of epidemic outbreaks that have been traced to infected milk. Swithinbank and Newman have pointed out that up to 1901 there have been no less than 330 such epidemics which have been traced to milk, and there are unquestionably many more where the responsibility has not been fixed. Of these, 195 were epidemics of typhoid fever, 99 of scarlet fever, and 36 of diphtheria. From these figures it is evident that some form of supervision is necessary in order to guard against the constant repetition of these epidemic outbreaks.

The methods for the examination of water have been developed and tested with great care, and are in the main satisfactory. The adoption of the so-called standard methods in the different laboratories of the country has given the semblance of uniformity to the work, and has made the results of one laboratory comparable with those of another. Such has not been the case with milk examination. In the various laboratories working upon this subject, widely different methods are employed, with the effect that the results of but few laboratories can be regarded as comparable. Differences in the composition of the culture media employed, variations in the methods and amount of dilution, and in the temperature and duration of incubation, as well as minor differences, all tend to produce results which are valueless for comparison.

It is the purpose of this paper to suggest that the methods which are found reasonably satisfactory in the sanitary examination of water should be modified and extended in such ways as may be necessary, and adopted by the members of this Association as "standard" procedures. This is the more necessary at the present time because of the ordinances and legal regulations governing the sale of milk.

To show the variations which may be brought about in the results by the different methods of procedure which I have mentioned, I have made a careful comparison of the number of bacteria developing at body and room temperatures in a large number of samples of milk. The body temperature count was made at the expiration of 24 hours; the room temperature, on three successive days; that is, after 24, 48, and 72 hours.

Without giving detailed results, it may be stated that in about 30 per cent of the cases the number of bacteria developing at body temperature was in excess of those developing at room temperature. If we may assume that the best incubation temperature for inspection purposes is the one giving us the highest count, then it is apparent that incubation should be carried on at 20° C. I am indebted to Dr. Slack for data confirming these results. Since, however, the quickness with which the results may be obtained is oftentimes an important factor, the body temperature count would probably be the more useful, although not generally giving as high results. In any case, it should be borne in mind that the count does not represent the actual total number of bacteria present, but merely those which will develop readily under the existing conditions. For the purpose of comparison, therefore, it is only necessary to establish a definite incubation temperature and duration period.

A large number of samples of milk have also been examined by plating upon ordinary nutrient agar and nutrient lactose agar to determine whether the presence of milk sugar was favorable to an increased development of the bacteria. In this case almost exactly 50 per cent of the plates made with the lactose agar gave results higher than corresponding plates with ordinary agar, and *vice versa*, indicating that it makes no difference which of these media is used for routine work.

More important perhaps are some observations upon the effect of keeping samples of milk before plating. My own experience, extending over several years, has shown that keeping milk packed in ice—that is, at a temperature approaching 32° F.—makes very little difference in the number of bacteria developing on agar plates in the first 24 hours. At the temperature of the ordinary ice-chest—that is, about 50° F.—it has been repeatedly shown that the number of bacteria, especially of the putrefactive bacteria, increases with considerable rapidity. Again I am indebted to Dr. Slack for confirmatory evidence. He found, in 29 samples kept for four hours in a collecting case at 34° F., that the number of bacteria increased slowly in eight samples, decreased slowly in 19, and remained constant in two. Eleven samples kept for seven hours showed slight increase in four, and slight decrease in six, while 11 samples kept for 24 hours showed slight increase in four, and slight decrease in seven. He has also shown that when samples were collected without special care as to temperature were plated, and then kept for from four to six hours at 34° F., plates again gave a slight decrease in about 75 per cent of the samples.

It is not the purpose of this paper to suggest methods which should be regarded as "standard." I have merely desired to call attention to the need for such methods and to show by a few illustrations why results obtained by procedures at present in use in different laboratories are discordant.

If it is agreeable to the section, I should like to recommend that a committee be appointed to consider all phases of laboratory procedure in the sanitary examination of milk, and to compare, for example, the effect of dilution in various ways and different degrees, to compare the different media, the temperature and duration of incubation, the effect of keeping the sample under different conditions, and such other factors as will readily suggest themselves.

*DISCUSSION OF PAPERS.

DISCUSSION ON THE PAPER OF DR. KLOTZ.

Dr. F. F. Wesbrook, of Minneapolis, Minnesota.—Dr. Klotz's paper recalls to my mind an experience which we had in our laboratory some years ago, when the methods for exact differentiation were not so satisfactory as they are at the present time. In our experiments carried on by Dr. McDaniel it was found that the cultural characteristics of certain bacteria derived from the body of a fetus were remarkably like those of B. typhosus. The mother was suffering from typhoid fever when abortion took place. An examination of her blood gave a well-marked Widal reaction. Her blood was not at that time studied culturally, as it would be now, but there was little doubt in our minds that typhoid infection was present. There were several varieties of bacilli obtained from the fetus within a few hours after expulsion. From the liver a bacillus, which resembled in practically all respects the B. typhosus, was isolated. Its motility and morphology were identical with the typhoid bacillus. With dextrose, acid was produced without gas formation, and with lactose neither acid nor gas was formed. Its other sugar reactions were negative, and in every respect it seemed to correspond culturally with the B. typhosus. In addition, it reacted with the blood of the mother and with the blood of the child in dilutions of 1:100 of the dried blood. But some weeks afterward, to our great surprise, this pure culture began to produce gas in dextrose media, and we were at a loss to know how to classify it. I presmue it would be regarded now as belonging to the class of paratyphoid, but at that time I was looked upon as rather heterodox, when I wondered whether from its development in the liver of the child the glycogen had not modified the typhoid bacillus so as to lead it to ferment dextrose with the formation of gas as well as of acid. Force of circumstances prevented Dr. McDaniel from carrying on the work, and the organism was unfortunately lost. This case was reported in the *Journal of the American Medical Association*, February, 1902.

Dr. Theobald Smith, of Boston, Mass.—Dr. Klotz's paper is very interesting and suggestive. Much is still to be done to determine how much bacteria may vary under changing conditions. My own observations of a large number of cultures of B. coli and its variants lead me to regard the gas production and gas formula as a very stable function. I have never seen any change in cultures on my hands, although I have kept them for years. It seems, however, as brought out by Dr. Klotz and in an earlier paper by Mr. Fuller, that the gas production of the colon bacillus may be temporarily aberrant when the organisms have been in an untoward environment. Whether the characters can be wholly altered by a persistence of such environment is a question of very great importance.

DISCUSSION ON THE PAPER OF MR. E. C. LEVY.

Mr. Richard B. Dole, of Washington, D. C.—I should like to ask Mr. Levy if in using the silicate standard he follows the same scale chemically; that is, can he use a candle through the same depth of liquid in different dilutions as with the silicate standard?

MR. LEVY (closing the discussion).—I made between 100 and 200 tests, and of all degrees, and they all agreed. Every box of it seemed perfectly uniform. Each laboratory worker can make his own standard, and he can duplicate it at any time. Each box contains 200 grams, which is enough to last any one individual a lifetime, and it costs only twenty-five cents. It is an easy method of doing a very difficult thing.

DISCUSSION ON THE PAPER OF MR. WHIPPLE AND MR. MAYER

MR. GEORGE W. FULLER, of New York.—It makes a good deal of difference as to the solubility of the normal carbonates of lime and magnesia, what the alkalinity of a water is, and what the associated salts are.

In some test work done at Cincinnati eight or ten years ago there was an attempt made with certain devices to coagulate clay bearing water by adding an excess of lime water. This excess of caustic lime was so great that we made a great many analytical studies to determine how much of the carbonate of lime was left in the water. It was only three to five parts per million. A number of other studies make it plain to me that the amount of associated constituents is quite a factor.

In regard to the alkalinity due to the carbonates of lime and magnesia which remain in water after boiling, the point is well taken by Mr. Whipple that there is alkali obtained from the glass vessels that are frequently used. I have used platinum, and found that the temporary hardness of water is diminished slowly by boiling; but it can be diminished much below 30 to 35 parts per million which one sees in dictionaries on solubilities and in textbooks particularly on water softening. Speaking generally, I believe it is difficult to reason satisfactorily from the results obtained with one water or one set of conditions to those of another, in which the salts of lime and magnesia, are different in kind and amount, and in which other bases are present in different combinations and amounts.

MR. WHIPPLE (closing the discussion).—In reading our paper I omitted a few things in order not to exceed the time limit, and among them this very point. We tried the effect of the presence of calcium sulphate on the solubility of calcium carbonate, and found that it made quite a difference in the result.

MR. R. S. WESTON, of Boston.—I should like to ask Mr. Whipple if he made any experiments to show the relative speeds of reaction spoken of with magnesium and calcium, and whether magnesia was precipitated with the lime, after agitation?

MR. WHIPPLE.—We made no experiments in which the water was kept in agitation during the progress of the reaction.

CONSTITUTION OF THE LABORATORY SECTION OF THE AMERICAN PUBLIC HEALTH ASSOCIATION.

General Organization.

1. This organization shall be known as the Laboratory Section of the American Public Health Association.

2. It shall consist of an Administrative Division for the conduct of business and policies of the Section, and a Technical Division for the promotion of the scientific objects of the Section.

3. The Administrative Division shall consist of the Council, on whose members all the business of the Section shall devolve.

4. The Technical Division shall consist of the general body of the members of the Section organized into Committees.

The Chairmen of these Committees shall together form the Committee on Technical Progress, over which the Section Chairman shall preside. Upon this Committee shall devolve the responsibility for the scientific work and development of the Section.

Objects.

5. The presentation and discussion of papers bearing upon the theory and practice of bacteriology, chemistry, etc., as applied to the advancement of sanitary science.

6. The co-operation of members of the Technical Committees for the solution of definite problems concerning such work.

7. The definite co-operation of the Section, as representing technical laboratory work, with the Association as representing also executive work, for the promotion of uniformity, co-ordination, and progress in public health theory and practice.

Membership.

8. There shall be but one class of membership, the active. Bacteriologists, chemists, and other laboratory workers who are members of the American Public Health Association and who have been or are in the active practice of their professions are alone eligible.

9. Election to membership requires that applications be endorsed by three members of the Section; placed in the hands of the Section Secretary; then approved by the Section Committee on Membership; and finally confirmed by a two-thirds vote of the Section members present at the meeting when they are voted upon.

10. Failure to maintain membership in the American Public Health Association, or absence from three consecutive meetings of the Section shall be regarded

as resignation from the Section; but the presentation to the Committee on Membership of a reasonable excuse in writing for such absence, or the contribution of a paper, shall be equivalent to attendance at that meeting to which the paper or excuse shall be presented.

DUTIES OF OFFICERS, COUNCIL, AND COMMITTEES.

11. The officers of the Section shall be a Chairman, a Vice-Chairman, a Secretary and a Recorder.

12. Five advisory members, not officers of the Section, one of whom shall be the retiring Chairman, shall be elected to form, with the officers of the Section, the Section Council.

13. The duties of the Section Chairman shall be: To preside at all meetings of the Section; to preside at all meetings of the Council; to preside at all meetings of the Committee on Technical Progress.

To act as a member of the Committee on Program, the Committee on Publication and the Committee on Membership.

14. The duties of the Section Vice-Chairman shall be: To preside at meetings of the Section and Council in the absence of the Chairman.

To act as Chairman of the Committee on Program.

To act as member of the Council, of the Committee on Membership, and of the Committee on Publications.

15. The duties of the Secretary shall be: To conduct all the correspondence of the Section (except that relating to papers) and to have custody of the same.

To keep a careful record of the meetings of the Section and Council, and to report to the Section Chairman when the time allotted for papers and their discussion has expired.

To act as Chairman of the Committee on Membership.

To be the medium of communication with the American Public Health Association regarding membership.

To receive and have custody of all applications for membership.

Upon receipt of a properly endorsed application for membership, the Secretary shall file the same and submit it to the Committee on Membership. If not approved, the applicant shall be so notified; but if approved, the Secretary shall submit it to the Section Chairman for the vote of the Section. He shall also prepare and maintain a correct list of the members of the Section.

To preside at meetings of the Section or Council in the absence of the Section Chairman and Vice-Chairman.

To act as a member of the Committee on Publication and of the Committee on Program.

The Secretary shall be elected for a period of two years.

16. The duties of the Recorder shall be:

To receive and be the custodian of all correspondence and papers relating to articles submitted for presentation to the Section, and to carry out the rules relating to these matters. Upon receipt of a paper or an abstract of a paper, he shall acknowledge and file it for presentation to the Program Committee.

To have charge of all communications with the American Public Health Association regarding the publication of papers.

To preside at meetings of the Section or Council in the absence of the other officers.

To act as Chairman of the Committee on Publication.

To act as a member of the Committee on Program and of the Committee on Membership.

The Recorder shall be elected for a period of two years.

17. The duties of the advisory members shall be

To attend all meetings of the Section and Council and to constitute with the Section officers the Committees on Publication, Program, and Membership.

18. The first meeting of the newly elected Council shall be held before the last session of the regular Section meeting and they shall then prepare for presentation at the last session, a list of the Chairmen and Members of the Technical Committees to be elected for the ensuing year; they shall also at this time report on any other business of importance.

19. A quorum of the Council shall consist of five members, one of whom shall be an officer of the Section.

CONDUCT OF SECTION MEETINGS.

20. The Committee on Program shall endeavor to secure suitable scientific and technical material for the meetings and shall arrange the order of business for each session.

21. The order of business prepared by the Committee on Program shall not be departed from except by the vote of the Section.

22. The election of a Nominating Committee of five to suggest officers for the ensuing year shall be the first item of business transacted at the first session of the regular meeting. This Committee shall be nominated and elected from the floor. The Committee shall confer at once and report to the Section Chairman one name for each office which is to be vacated. The Secretary and Recorder shall not vacate office at the same time.

23. Upon receipt of this report, the Section shall cast ballots upon the names proposed. Any names rejected shall be replaced by nomination and election from the floor. Such nomination and the election in any case may not take place except as the first order of business of the second session.

24. Ten minutes' time shall be given the reader of each paper and it shall be the duty of the Chairman to announce the end of the allotted time and to prohibit further reading except by motion and vote from the floor. No member shall be given more time at his own request. Any paper refused by the Program Committee may be presented after the regular order of business and program

has been carried out, but all papers presented, whether accepted by the Program Committee or not, shall be passed upon by the Committee on Publication before final acceptance for publication.

25. The time of discussion shall not be limited, but no one member shall occupy more than five minutes consecutively. A motion that the discussion be closed shall always be in order during any discussion, but shall require a two-thirds vote to be sustained.

26. Every member presenting a paper to the Section shall furnish the Recorder with an epitome in which shall be expressed in less than three hundred words the problem attacked and the conclusions reached. This epitome shall be furnished to the Recorder not later than thirty days before the meeting at which the paper is to be read.

THE TECHNICAL COMMITTEES.

27. The Technical Committees shall consist of not more than five members each. The number and character of these committees shall be proposed and the Chairmen and members nominated from year to year by the Council before the end of the meeting at which said Council is elected. The deliberations of the Technical Committees shall embrace the following subjects amongst others:

The Bacteriology and Chemistry of Water.
The Bacteriology and Chemistry of Milk.
The Bacteriology and Chemistry of Food and Drugs.
The Bacteriology and Chemistry of Diagnostic Procedures and Results.
The Bacteriology and Chemistry of Human Pathological Processes.
The Bacteriology and Chemistry of Comparative Pathological Processes.
The Bacteriology and Chemistry of Remedial and Preventive Measures.

28. The Technical Committees shall deal with chemistry and bacteriology as they may bear on public health, and each committee shall report annually to the Section progress in research. The reports shall be deposited with the Recorder and shall be treated in the same manner as papers contributed to the Section.

29. The Chairman of each Committee shall hold himself in readiness to receive signed questions in writing on the subject-matter of his Committee from any member of the Association or Section, and to answer the same in his annual report to the Section.

30. Any committee of the Section except the Council may add to itself advisory members, not necessarily members of the Association or Section; but these advisory members shall have no vote in such committee nor may they sign its report.

31. A regular meeting of the Section shall consist of the regular session or sessions of the Section held in conjunction with the annual meeting of the American Public Health Association. A special session of the Section shall be any continuous assembly of a quorum of the Section, called by the Section Chairman, the order of business having been arranged by the Committee on Program, and an

officer of the Section occupying the chair; provided also that due notice of such session shall have been given to the section members at least one month previously.

32. A quorum of the Section shall consist of fifteen members, one of whom shall be an officer of the Section.

33. This constitution may be amended in any particular by a two-thirds vote of the members present at a regular meeting, provided the proposed amendment shall have been submitted in writing and read to the Section at a previous regular meeting of the Section, and approved by the Council before its submission to the Section for action. But amendments to the constitution, if made in writing and read at any regular session of the Section, shall become a part of the constitution if accepted by a unanimous vote of the Section at the next regular session.